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Original Research

Higher Levels of Plasma Fetuin-A, Nrf2, and Cytokeratin 18 in Patients with Hashimoto's Disease

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Abstract

Objectives: Fetuin-A is a protein that exhibits proatherogenic, pro-inflammatory, and anti-inflammatory effects with increased insulin resistance and adipocyte dysfunction. The nuclear factor erythroid 2-related factor (Nrf2) is a transcription factor that is crucial for protecting cells against oxidative damage. As a cell death product, cytokeratin 18 (CK18) levels increase during necrosis and apoptosis of both normal and tumor cells. We analyzed the plasma levels of three biomarkers based on the hypothesis that they might be related to some pathophysiological pathways in Hashimoto's disease.

Methods: We compared 34 female patients with overt hypothyroidism due to Hashimoto's disease (Group 1) with 34 age-matched healthy females (Group 2). For comparison, plasma levels of thyroid-stimulating hormone (TSH), fetuin-A, Nrf2, and CK18 were measured in all participants.

Results: In group 1, the mean TSH levels (31.4±15.3) were significantly higher than those in group 2 (2.6±1.0) ($p<0.001$). The levels of mean fetuin-A (606.7±34.2) and Nrf2 (1.3±0.6) were found to be significantly higher in group 1 than in group 2 (440.0±34.2 vs. 0.7±0.2) ($p<0.001$ for both). CK18 levels in group 1 (0.36±0.13) were also significantly higher than in group 2 (0.26±0.16) ($p=0.020$). A significant correlation was observed between TSH levels and fetuin-A ($r=0.401$, $p=0.001$).

Conclusion: Increased levels of fetuin-A, Nrf2, and CK18 may be a consequence or cause of the pathophysiological pathways of Hashimoto's disease. The clinical significance of increased levels of these biomarkers requires further investigation.

Keywords: Cytokeratin18, fetuin-A, Hashimoto's disease, nuclear factor erythroid 2-related factor

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Fetuin-A is a glycoprotein expressed in a variety of cell types, predominantly in embryonic cells and adult hepatocytes, and to a lesser extent in adipocytes, monocytes, and pancreatic beta cells. Due to its ability to bind to so many receptors, fetuin-A is involved in many physiological and pathological processes.[1] In addition to its proven effects on bone remodeling, calcium metabolism, inhibition of the insulin signaling pathway and adipocyte dysfunction, fetuin-A also exerts pro-atherogenic, pro-inflammatory, and anti-inflammatory effects.[2-6] In addition, apart from its potential use as a diagnostic marker and a prognostic predictor, there are ongoing promising studies regarding fetuin-A being a therapeutic target in clinical practice.[7]

The redox balance of cells is tightly regulated to meet physiological needs by maintaining the correct balance between pro-oxidants and reductants. The nuclear factor erythroid 2-related factor (Nrf2) is a transcription factor that encodes antioxidant, anti-inflammatory, and detoxi-

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fying proteins. The Nrf2/antioxidant-responsive element pathway is therefore crucial for protecting cells against oxidative damage.[8] In light of this, chronic diseases associated with oxidative stress and inflammation are expected to benefit from Nrf2 activators.[9]

Cytokeratins (CKs) are a group of structural proteins that are derived from keratin-containing intermediate filaments in the cytoplasm of epithelial cells. These proteins consist of at least 20 unique gene products that fall into two categories according to their isoelectric pH value: The acidic type I group (CK9–CK20) and the neutral to basic type II group (CK1–CK8).[10] Cytokeratin 18 (CK18) has a wide variety of functions in epithelial cells, such as structuring the cytoplasm, protecting cells against external pressure, and maintaining normal mitochondrial functions.[10,11] CK18 is also involved in cellular processes such as mitosis, cell signaling, and apoptosis. There is strong evidence that blood levels of soluble and fragmented CK18 can be used as biomarkers of apoptotic cell death.[12] In addition, CK18 has been used for many years as an epithelial marker in diagnostic histopathology and plays a key role in the behavior of tumor cells.[13-15]

Hashimoto's thyroiditis, also known as Hashimoto's disease, is an autoimmune disorder characterized by the gradual destruction of thyroid follicles.[16] Inflammatory reactions induced by the immune system, oxidative stress, and apoptotic processes play a significant role in thyroid follicular damage.[17,18] Hypothyroidism has also been linked with an increased level of insulin resistance (IR) and a higher incidence of atherosclerotic cardiovascular diseases.[19,20] In this study, we investigated fetuin-A, Nrf2, and CK18 levels in patients with Hashimoto's disease. We hypothesized that relevant biomarkers may be associated with some pathophysiological pathways in Hashimoto's disease as discussed above.

Methods

This study included 34 outpatient women with overt hypothyroidism due to Hashimoto's disease and who did not receive levothyroxine replacement therapy (group 1). Considering the significant increase in the prevalence of Hashimoto's thyroiditis among females, the study focused primarily on females. An age-matched and healthy group of 34 women (group 2) was recruited as a control group. Study participants were selected based on the date of their hospital admission. Those who were pregnant, who had diabetes, hypertension, dyslipidemia, organ failure, rheumatological diseases, and malignancies were excluded from the study. In addition, participants with inflammation, such as acute or chronic infections, those with a body mass index >30 kg/m^2, and those who used supportive antioxidants such as resveratrol, curcumin, and sulforaphane, were excluded from the study. Hashimoto's thyroiditis was diagnosed by a combination of serum antibodies to thyroid antigens (mainly thyroperoxidase and thyroglobulin) and thyroid sonography.[21] The presence of overt hypothyroidism was defined as thyroid-stimulating hormone (TSH) levels >10 uIU/mL or TSH levels >8 uIU/mL with low free T4 (fT4) and/or free T3 (fT3) levels.

Following an overnight fast, blood samples were collected between 9.00 and 10:00 in the morning to avoid circadian rhythm effects. The measurements of TSH, fT3, and fT4 were done using the Atellica Solution Immunoassay Analyzer (Siemens Healthcare Diagnostics Inc. Erlangen, Germany). The serum samples collected from the participants were centrifuged and stored at −80 °C until analysis was performed. All samples were dissolved at room temperature before measurements, and samples were then analyzed with commercial ELISA kits on the same day. The level of fetuin-A was measured using an ELISA kit (Bioassay Technology Laboratory, Co. Ltd., Shanghai, China) with a standard measurement range of 10 µg/mL to 4000 µg/mL and a sensitivity of 5.12 µg/mL. The level of Nrf2 was measured using an ELISA kit (Bioassay Technology Laboratory, Co. Ltd., Shanghai, China) with a standard measurement range 0.2 ng/mL–70 ng/mL and a sensitivity of 0.08 ng/mL. The level of CK-18 was measured using an ELISA kit (Bioassay Technology Laboratory, Co. Ltd., Shanghai, China) with a standard measurement range of 0.05 ng/mL–20 ng/mL and a sensitivity of 0.023 ng/mL.

The study protocol was approved by the clinical research Ethics Committee with number 18/07/14. Written informed consent was obtained from all subjects. The study was performed in accordance with the Declaration of Helsinki.

Statistical Analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 22.0 (SPSS Inc., US). Data are expressed as numbers and percentages if categorical, or mean±SD if numerical. We used the Kolmogorov–Smirnov test to determine whether the continuous variables had a normal distribution. The independent sample t-test or the Mann–Whitney U-test was used for normal and abnormal continuous data, respectively. Pearson's correlation coefficient was used for the evaluation of correlations between the parameters. A $p<0.05$ was considered as statistically significant.

Results

There were no significant differences between the two

groups in terms of mean age, body mass index, fasting plasma glucose, creatinine, and alanine aminotransferase levels. In group 1, the mean TSH levels (31.4±15.3 μIU/mL) were significantly higher than those in group 2 (2.6±1.0) (p<0.001). The levels of mean fetuin-A (606.7±34.2 μg/mL) and Nrf2 (1.3±0.6 ng/mL) were found to be significantly higher in group 1 than in group 2 (440.0±34.2 μg/mL vs. 0.7±0.2 ng/mL) (p<0.001 for both). In addition, CK-18 levels in group 1 (0.36±0.13 ng/dL) were also significantly higher than in group 2 (0.26±0.16 ng/dL) (p=0.020). Comparisons of the clinical and biochemical characteristics of the groups are presented in Table 1.

The results of the Pearson correlation analysis are summarized in Table 2. A significant correlation was observed between TSH levels and fetuin A (r=0.401, p=0.001). Despite this, there was no significant correlation between TSH levels and Nrf2 (r=0.168, p=0.171) or CK18 levels (r=0.228, p=0.061).

Table 1. Comparison of the clinical and biochemical data of the groups

Data	Group 1 (n=34)	Group 2 (n=34)	p
Age (years)	31.1±5.8	29.5±6.3	0.06
BMI (kg/m^2)	25.8±2.6	25.2±2.7	0.37
FPG (mg/dL)	89.7±10.1	85.6±6.9	0.06
Creatinine (mg/dL)	0.71±0.10	0.70±0.06	0.62
ALT (U/L)	16.8±6.2	18.5±10.3	0.80
TSH (μIU/mL)	31.4±15.3	2.6±1.0	<0.001
Body water (%)	47.8±5.2	52.0±4.2	<0.001
Lean mass (%)	65.2±7.1	71.2±5.6	<0.001
Body fat (%)	34.7±7.1	28.5±5.3	<0.001
Fetuin-A (μg/mL)	606.7±34.2	440.0±34.2	<0.001
Nrf2 (ng/mL)	1.3±0.6	0.7±0.2	<0.001
CK 18 (ng/dL)	0.36±0.13	0.26±0.16	0.020

ALT: Alanine transaminase; BMI: Body mass index; CK 18: Cytokeratin 18; FPG: Fast blood glucose; Nrf2: Nuclear factor erythroid 2-related factor; TSH: Thyroid stimulating hormone; significant p-values between group 1 and 2 are highlighted in bold.

Table 2. Analysis of pearson correlations between parameters and TSH

Parameters	Nrf2	CK 18	Fetuin-A
TSH	r=0.168	r=0.228	r=0.401
	p=0.171	p=0.061	p=0.001
Nrf2		r=0.287	r=0.468
		p=0.018	p=0.000
CK 18			r=0.573
			p=0.000

CK 18: Cytokeratin 18; Nrf2: Nuclear factor erythroid 2-related factor; TSH: Thyroid stimulating hormone; Significant p-values are highlighted in bold.

Discussion

In this study, we hypothesized that fetuin-A, Nrf2, and CK18 might be associated with the pathophysiological pathways of Hashimoto's thyroiditis. It was found that all three biomarkers were significantly higher in Hashimoto's patients than in healthy individuals.

Fetuin-A is a multifunctional glycoprotein that is predominantly (more than 95%) synthesized in the adult liver. It is also synthesized in small amounts in certain cells, such as adipocytes, monocytes, and macrophages. There is currently a growing number of biological functions attributed to fetuin-A, which is largely due to its interaction with a wide variety of receptors.[1] For a long time, it was believed that fetuin-A played a crucial role in preventing ectopic calcification by dissolving calcium and phosphorus in the serum.[2] However, high circulating levels of fetuin-A inhibit the insulin signaling pathways and cause adipocyte dysfunction. This may lead to IR and associated comorbid conditions such as impaired glucose tolerance, type 2 diabetes mellitus, obesity, non-alcoholic fatty liver disease, and hypertriglyceridemia.[3-5] The role of fetuin-A in the development of atherosclerotic cardiovascular disease is thought to be biphasic. Increased cardiovascular events have been associated with both elevated and decreased levels of fetuin-A. Elevated serum fetuin-A can play an atherogenic role, especially in the early stages of atherosclerosis, possibly due to underlying IR and adipocyte dysfunction. Alternatively, a decrease in fetuin-A levels may worsen atherosclerosis by reducing its function as an inhibitor of vascular calcification.[6] Thyroid hormones can show both insulin agonist and antagonist effects depending on different target tissues. In euthyroid condition, these opposite effects are balanced. However, an imbalance of thyroid hormones can disrupt this balance and alter carbohydrate metabolism.[19] A number of studies conducted on rats and humans with overt hypothyroidism have demonstrated impaired peripheral glucose uptake as a result of IR.[22-25] In addition, a small number of studies specifically conducted on Hashimoto's patients have also reported increased IR. However, the pathophysiology of increased IR in hypothyroid conditions, both autoimmune and other causes, is not fully understood. Some of the proposed pathophysiological mechanisms include impaired GLUT4 translocation to the cell surface, a reduction in blood flow to fat tissues and muscles, dysregulation of leptin action at the hypothalamus, and a decreased glycogen synthesis rate.[22-26] We found a significant increase in fetuin-A levels among Hashimoto patients compared with the control group. The elevated level of fetuin-A may be one of the factors contributing to the pathophysiology of IR in this patient group.

Nrf2 is a transcription factor that encodes antioxidant, anti-inflammatory, and detoxifying proteins in response to oxidative stress.[8] Previous research has shown that Nrf2 has protective effects on the development and exacerbation of various inflammatory conditions.[9] A number of animal studies have shown that activating Nrf2 has positive effects on smoking-induced lung inflammation, allergic asthma, diabetes development, organ damage in sickle cell anemia, and experimental sepsis.[27-32] In addition, the Nrf2 activator dimethyl fumarate is approved for the treatment of relapsing forms of multiple sclerosis.[33] An interesting paradox associated with Nrf2 activation is its ability to promote the proliferation of cancer cells, increase metastasis risk, and produce resistance to chemotherapy.[9] Although Nrf2 has been well studied in other tissues, little is known about its effects on the thyroid gland pathophysiology.[34] It is important to note that, unlike in many other tissues, the thyroid gland requires a continual supply of reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2), to function properly and synthesize its hormones. To protect follicular cells from oxidative damage, adequate expression of antioxidant and cytoprotective enzymes is also essential. As in other tissues, Nrf2 plays an important role in regulating antioxidant and cytoprotective genes in the thyroid gland.[35] Despite the lack of sufficient data to date, it appears that genetically induced Nrf2 overactivation results in thyroid gland enlargement.[36] In addition, previous studies have indicated that Nrf2 is highly expressed in papillary thyroid carcinoma.[37] In general, it has been proposed that activating the Nrf2 pathway supports cancer cell survival via sustained antioxidant responses and crosstalk with proliferation pathways.[35] In a recent study, Nrf2 downregulation was reported to reduce the growth and proliferation of anaplastic thyroid tumor cells and improve chemotherapy response.[38] There is evidence that high ROS levels play a role in Hashimoto's and Graves' disease pathogenesis and exacerbation.[39] However, little is known about the role of Nrf2 in autoimmune thyroid diseases. Considering its antioxidant effects, it is thought that Nrf2 activation may have beneficial effects in autoimmune thyroid diseases.[35] To the best of our knowledge, there was no study that evaluated the Nrf2 level in patients with Hashimoto's disease. In our study, we found that Nrf2 level was significantly higher in patients with Hashimoto's disease compared to the control group. This may be a physiological response to increased oxidative stress.

CK18 is highly expressed in epithelial cells, and its levels increase following their destruction.[10] In both normal and tumor cells, increased CK18 levels have been shown to be an indirect indicator of apoptosis and necrosis.[12,13] In addition, some evidence suggests that CK18 levels may serve as an indirect indicator of chemotherapy response in cancer patients.[14] The expression of CK18 in normal thyroid glands has also been demonstrated.[10] In patients with papillary thyroid cancer, increased levels of CK18 have been demonstrated; however, it is unknown how this changes in patients with Hashimoto's disease.[15] In consideration of the fact that apoptosis plays an important role in thyroid follicular damage in Hashimoto patients, we assessed CK18 levels as a cell death product in our patients.[16] Our patient group had significantly higher CK18 levels than the controls. An elevated CK18 level may indicate structural deformation of the thyroid gland due to chronic inflammation due to autoimmune damage. To better understand, the role of CK 18 in Hashimoto's thyroiditis, additional comprehensive studies are required.

Study Limitations

The study has several limitations that should be noted. First, only female patients were evaluated. In light of the significantly higher prevalence of Hashimoto's thyroiditis in females, this study focused primarily on females. The evaluation of male patients from this perspective may be beneficial in future studies. Second, all of our patients with Hashimoto's thyroiditis displayed overt hypothyroidism. Therefore, our findings may be related to pathological mechanisms caused by hypothyroidism independent of Hashimoto's disease. A comparison with euthyroid patients with Hashimoto's disease may be useful for clarifying this issue. Third, it has been demonstrated in several studies that both fetuin-a and Hashimoto's thyroiditis are associated with increased IR.[3-5,22-26] However, in the present study we were unable to assess IR. The fourth limitation is that thyroid autoantibodies were unable to be compared with the biomarkers examined. Finally, since we included healthy individuals as the control group, we were unable to analyze the levels of these biomarkers in other thyroid diseases characterized by thyroid follicular damage.

Conclusion

All three serum markers were significantly increased in Hashimoto patients compared to controls. Increased levels of fetuin-A, Nrf2, and CK18 may be a consequence or cause of the pathophysiological pathways. Further research may uncover additional evidence of elevated levels of these biomarkers in this patient group. In this respect, these biomarkers may provide an effective tool for diagnosing and monitoring disease, as well as for developing novel therapeutic approaches.

Disclosures

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References

1. Chekol Abebe E, Tilahun Muche Z, Behaile T/Mariam A, Mengie Ayele T, Mekonnen Agidew M, Teshome Azezew M, et al. The structure, biosynthesis, and biological roles of fetuin-A: a review. Front Cell Dev Biol 2022;10:945287. [CrossRef]
2. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, et al. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. J Clin Invest 2003;112:357–66. [CrossRef]
3. Mukhopadhyay S, Mondal SA, Kumar M, Dutta D. Proinflammatory and antiinflammatory attributes of fetuin-a: a novel hepatokine modulating cardiovascular and glycemic outcomes in metabolic syndrome. Endocr Pract 2014;20:1345–51. [CrossRef]
4. Dasgupta S, Bhattacharya S, Biswas A, Majumdar SS, Mukhopadhyay S, Ray S, et al. NF-kappaB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. Biochem J 2010;429:451–62. [CrossRef]
5. Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. J Am Soc Nephrol 2010;21:406–12. [CrossRef]
6. Mori K, Emoto M, Inaba M. Fetuin-A and the cardiovascular system. Adv Clin Chem 2012;56:175–95. [CrossRef]
7. Chekol Abebe E, Tilahun Muche Z, Behaile T/Mariam A, Mengie Ayele T, Mekonnen Agidew M, Teshome Azezew M, et al. Role of fetuin-A in the pathogenesis of psoriasis and its potential clinical applications. Clin Cosmet Investig Dermatol 2022;15:595–607.
8. Lee JM, Johnson JA. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J Biochem Mol Biol 2004;37:139–43.
9. Bocci V, Valacchi G. Nrf2 activation as target to implement therapeutic treatments. Front Chem 2015;3:4. [CrossRef]
10. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology 2002;40:403–39. [CrossRef]
11. Kumemura H, Harada M, Yanagimoto C, Koga H, Kawaguchi T, Hanada S, et al. Mutation in keratin 18 induces mitochondrial fragmentation in liver-derived epithelial cells. Biochem Biophys Res Commun 2008;367:33–40. [CrossRef]
12. Sheard MA, Vojtesek B, Simickova M, Valik D. Release of cytokeratin-18 and -19 fragments (TPS and CYFRA 21-1) into the extracellular space during apoptosis. J Cell Biochem 2002;85:670–7. [CrossRef]
13. Ueno T, Toi M, Linder S. Detection of epithelial cell death in the body by cytokeratin 18 measurement. Biomed Pharmacother 2005;59 Suppl 2:S359–62. [CrossRef]
14. Olofsson MH, Ueno T, Pan Y, Xu R, Cai F, van der Kuip H, et al. Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. Clin Cancer Res 2007;13:3198–206. [CrossRef]
15. Yan G, Zhou Y, Wu H, Xun H. Diagnostic value of serum cytokeratin 18, carcinoembryonic antigen, and thyroglobulin in patients with papillary thyroid carcinoma. Clin Lab 2021;67. [CrossRef]
16. Ralli M, Angeletti D, Fiore M, D'Aguanno V, Lambiase A, Artico M, et al. Hashimoto's thyroiditis: an update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential malignant transformation. Autoimmun Rev 2020;19:102649.
17. Di Dalmazi G, Hirshberg J, Lyle D, Freij JB, Caturegli P. Reactive oxygen species in organ-specific autoimmunity. Auto Immun Highlights 2016;7:11. [CrossRef]
18. Wang SH, Baker JR. The role of apoptosis in thyroid autoimmunity. Thyroid 2007;17:975–9. [CrossRef]
19. Brenta G. Why can insulin resistance be a natural consequence of thyroid dysfunction? J Thyroid Res 2011;2011:152850. [CrossRef]
20. Ichiki T. Thyroid hormone and atherosclerosis. Vascul Pharmacol 2010;52:151–6. [CrossRef]
21. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 2014;13:391–7. [CrossRef]
22. Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Panagiotakos DB, et al. Insulin action in adipose tissue and muscle in hypothyroidism. J Clin Endocrinol Metab 2006;91:4930–7.
23. Rochon C, Tauveron I, Dejax C, Benoit P, Capitan P, Fabricio A, et al. Response of glucose disposal to hyperinsulinaemia in human hypothyroidism and hyperthyroidism. Clin Sci (Lond) 2003;104:7–15. [CrossRef]
24. Cettour-Rose P, Theander-Carrillo C, Asensio C, Klein M, Visser TJ, Burger AG, et al. Hypothyroidism in rats decreases peripheral glucose utilisation, a defect partially corrected by central leptin infusion. Diabetologia 2005;48:624–33. [CrossRef]
25. Maratou E, Hadjidakis DJ, Kollias A, Tsegka K, Peppa M, Alevizaki M, et al. Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. Eur J Endocrinol 2009;160:785–90.
26. Peppa M, Koliaki C, Nikolopoulos P, Raptis SA. Skeletal muscle insulin resistance in endocrine disease. J Biomed Biotechnol 2010;2010:527850. [CrossRef]
27. Blake DJ, Singh A, Kombairaju P, Malhotra D, Mariani TJ, Tuder RM, et al. Deletion of Keap1 in the lung attenuates acute cigarette smoke-induced oxidative stress and inflammation. Am J Respir Cell Mol Biol 2010;42:524–36. [CrossRef]

28. Sussan TE, Gajghate S, Chatterjee S, Mandke P, McCormick S, Sudini K, et al. Nrf2 reduces allergic asthma in mice through enhanced airway epithelial cytoprotective function. Am J Physiol Lung Cell Mol Physiol 2015;309:L27–36. [CrossRef]
29. Uruno A, Furusawa Y, Yagishita Y, Fukutomi T, Muramatsu H, Negishi T, et al. The Keap1-Nrf2 system prevents onset of diabetes mellitus. Mol Cell Biol 2013;33:2996–3010. [CrossRef]
30. Yagishita Y, Fukutomi T, Sugawara A, Kawamura H, Takahashi T, Pi J, et al. Nrf2 protects pancreatic β-cells from oxidative and nitrosative stress in diabetic model mice. Diabetes 2014;63:605–18.
31. Keleku-Lukwete N, Suzuki M, Otsuki A, Tsuchida K, Katayama S, Hayashi M, et al. Amelioration of inflammation and tissue damage in sickle cell model mice by Nrf2 activation. Proc Natl Acad Sci U S A 2015;112:12169–74. [CrossRef]
32. Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, et al. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest 2006;116:984–95. [CrossRef]
33. Blair HA. Dimethyl fumarate: a review in relapsing-remitting MS. Drugs 2019;79:1965–76. [CrossRef]
34. Kumar H, Kim IS, More SV, Kim BW, Choi DK. Natural product-derived pharmacological modulators of Nrf2/ARE pathway for chronic diseases. Nat Prod Rep 2014;31:109–39. [CrossRef]
35. Chartoumpekis DV, Ziros PG, Habeos IG, Sykiotis GP. Emerging roles of Keap1/Nrf2 signaling in the thyroid gland and perspectives for bench-to-bedside translation. Free Radic Biol Med 2022;190:276–83. [CrossRef]
36. Ziros PG, Renaud CO, Chartoumpekis DV, Bongiovanni M, Habeos IG, Liao XH, et al. Mice hypomorphic for Keap1, a negative regulator of the Nrf2 antioxidant response, show age-dependent diffuse goiter with elevated thyrotropin levels. Thyroid 2021;31:23–35. [CrossRef]
37. Wang Z, Li J, Liu Z, Yue L. Nrf2 as a novel diagnostic biomarker for papillary thyroid carcinoma. Eur J Histochem 2023;67:3622.
38. Gong Z, Xue L, Wei M, Liu Z, Vlantis AC, van Hasselt CA, et al. The knockdown of Nrf2 suppressed tumor growth and increased the sensitivity to Lenvatinib in anaplastic thyroid cancer. Oxid Med Cell Longev 2021;2021:3900330. [CrossRef]
39. Burek CL, Rose NR. Autoimmune thyroiditis and ROS. Autoimmun Rev 2008;7:530–7. [CrossRef]