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. 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. 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. 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 detoxifying enzymes.
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). 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. 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. 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.

Hashimoto’s thyroiditis, also known as Hashimoto’s disease, is an autoimmune disorder characterized by the gradual destruction of thyroid follicles. Inflammatory reactions induced by the immune system, oxidative stress, and apoptotic processes play a significant role in thyroid follicular damage. Hypothyroidism has also been linked with an increased level of insulin resistance (IR) and a higher incidence of atherosclerotic cardiovascular diseases. 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/m², 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. 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 of 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n=34)</th>
<th>Group 2 (n=34)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.1±5.8</td>
<td>29.5±6.3</td>
<td>0.06</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8±2.6</td>
<td>25.2±2.7</td>
<td>0.37</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>89.7±10.1</td>
<td>85.6±6.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.71±0.10</td>
<td>0.70±0.06</td>
<td>0.62</td>
</tr>
<tr>
<td>ALT (µL/L)</td>
<td>16.8±6.2</td>
<td>18.5±10.3</td>
<td>0.80</td>
</tr>
<tr>
<td>TSH (µIU/mL)</td>
<td>31.4±15.3</td>
<td>2.6±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body water (%)</td>
<td>47.8±5.2</td>
<td>52.0±4.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>65.2±7.1</td>
<td>71.2±5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34.7±7.1</td>
<td>28.5±5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fetuin-A (µg/mL)</td>
<td>606.7±34.2</td>
<td>440.0±34.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nrf2 (ng/mL)</td>
<td>1.3±0.6</td>
<td>0.7±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CK 18 (ng/dL)</td>
<td>0.36±0.13</td>
<td>0.26±0.16</td>
<td>0.020</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nrf2</th>
<th>CK 18</th>
<th>Fetuin-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>r=0.168</td>
<td>p=0.171</td>
<td>r=0.401</td>
</tr>
<tr>
<td>Nrf2</td>
<td>r=0.287</td>
<td>p=0.018</td>
<td>r=0.468</td>
</tr>
<tr>
<td>CK 18</td>
<td>r=0.573</td>
<td>p=0.000</td>
<td></td>
</tr>
</tbody>
</table>

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.[18] 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. Previous research has shown that Nrf2 has protective effects on the development and exacerbation of various inflammatory conditions. A number of animal studies have shown that activating Nrf2 has protective effects on smoking-induced lung inflammation, allergic asthma, diabetes development, organ damage in sickle cell anemia, and experimental sepsis. In addition, the Nrf2 activator dimethyl fumarate is approved for the treatment of relapsing forms of multiple sclerosis. 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. Although Nrf2 has been well studied in other tissues, little is known about its effects on the thyroid gland pathophysiology. 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 (H₂O₂), 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. Despite the lack of sufficient data to date, it appears that genetically induced Nrf2 overactivation results in thyroid gland enlargement. In addition, previous studies have indicated that Nrf2 is highly expressed in papillary thyroid carcinoma. In general, it has been proposed that activating the Nrf2 pathway supports cancer cell survival via sustained antioxidant responses and crosstalk with proliferation pathways. In a recent study, Nrf2 downregulation was reported to reduce the growth and proliferation of anaplastic thyroid tumor cells and improve chemotherapy response. There is evidence that high ROS levels play a role in Hashimoto's and Graves' disease pathogenesis and exacerbation. 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. 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. In both normal and tumor cells, increased CK18 levels have been shown to be an indirect indicator of apoptosis and necrosis. In addition, some evidence suggests that CK18 levels may serve as an indirect indicator of chemotherapy response in cancer patients. The expression of CK18 in normal thyroid glands has also been demonstrated. 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. 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. 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 CK18 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. 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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Ethics Committee Approval: 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.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.


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