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# SERUM CARBOXYMETHYL-LYSINE AND SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS IN HYPERTHYROID AND HYPOTHYROID PATIENTS

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## Abstract

**Purpose.** The formation and accumulation of advanced glycation end products (AGEs) are enhanced with increased oxidative stress and inflammatory conditions. A hyperthyroid and hypothyroid state is associated with oxidative stress. This study aimed to evaluate skin AGE deposition, serum carboxymethyl-lysine (CML), and serum soluble receptor for AGEs (sRAGE) levels in hypothyroid and hyperthyroid patients.

**Methods.** A total of 203 subjects were included in this cross-sectional study. After excluding diabetes mellitus, 103 newly diagnosed hypothyroid patients, 50 newly diagnosed hyperthyroid patients, and 50 control (euthyroid) subjects were enrolled. All tests were done before beginning the appropriate treatment. Accumulated AGEs in the skin collagen were measured by skin autofluorescence (SAF) using an AGE Reader.

**Results.** SAF measurements were 1.82 ± 0.04, 1.80 ± 0.40, and 1.63 ± 0.30 arbitrary units for the hypothyroid, hyperthyroid, and euthyroid groups, respectively ($p = 0.04$). Serum CML levels were 8.2 ± 2.8, 10.2 ± 2.0, and 8.0 ± 3.3 ng/mL for the hypothyroid, hyperthyroid, and euthyroid groups, respectively ($p = 0.01$). sRAGE levels were similar between the groups. Serum thyroid-stimulating hormone and SAF measurements were positively correlated ($r = 0.25$, $p = 0.02$) in the hypothyroid group and negatively correlated in the hyperthyroid group ($r = -0.36$, $p = 0.04$). There was no correlation between CML and sRAGE levels.

**Conclusion.** SAF measurements are increased in both hypo- and hyperthyroid normoglycemic patients. Serum CML levels are increased in hyperthyroid patients. Hypo and hyperthyroid states might be associated with acceleration of AGE accumulation and may have a long term effect on metabolic memory.

**Keywords:** advanced glycation end products, skin autofluorescence, hypothyroidism, hyperthyroidism, soluble receptor for advanced glycation end products.

## INTRODUCTION

Advanced glycation end products (AGEs) are stable posttranslational modifications of proteins formed by non-enzymatic reactions with glucose and related metabolites (1). AGEs accumulate in tissues via aging, and their formation and accumulation are enhanced by increased oxidative stress (OS), inflammation, or chronic hyperglycemia. Therefore, AGEs are considered a marker of metabolic memory (2).

AGEs mediate their pathological effects by activating signaling cascades via the receptors for AGEs (sRAGE) (3). Binding to these receptors results in the release of cytokines and free radicals, which leads to oxidative damage to the endothelium, increased inflammation, smooth muscle cell proliferation, and atherosclerosis (4,5). Serum soluble RAGE (sRAGE) levels have decreased due to aging and chronic inflammatory diseases, including atherosclerosis, diabetes, and renal failure (6). Serum levels of glycoxidation products, such as N epsilon-(carboxymethyl)lysine (CML) and pentosidine, have been shown to correlate with the severity of complications in diabetic patients, including their risk of coronary heart disease, peripheral artery disease, stroke, and cardiovascular mortality (7-10).

AGEs can be easily measured in dermal tissue due to their fluorescent properties. Skin autofluorescence (SAF) is a non-invasive method for measuring AGE levels (11). Studies have shown that AGE levels in skin biopsies are closely related to SAF (12,13). SAF is associated with long-term cardiovascular complications and mortality in type 2 diabetes mellitus (DM) (14-17).

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Thyroid hormones are physiological mediators of oxidative stess and protein breakdown. Early studies indicated that thyroid status is associated with elevated OS and lipid peroxidation of tissue proteins (23,24). Recent studies have reported that high levels of AGEs and RAGEs can play a critical role in the pathogenesis of hyperthyroidism and its complications (25). Increased AGE levels are also reported in many diseases associated with inflammation and increased oxidative stress, such as inflammatory bowel diseases, rheumatoid arthritis, and chronic kidney failure (18-22).

Accumulated data suggest that thyroid hormone levels affect the formation of AGEs regardless of hyperglycemia. The question needs to be clarified, yet increased AGE production may play a role in the increased risk of cardiovascular disease in untreated hypothyroidism and hyperthyroidism.

We hypothesize that the hypothyroid and hyperthyroid states are associated with increased serum AGE levels and skin-accumulated AGE measurement. This study aims to evaluate serum CML, sRAGE levels, and skin AGE accumulation in patients newly diagnosed with primary hypothyroidism and hyperthyroidism.

## MATERIALS AND METHODS

### *Patient selection*

This cross-sectional study included 103 patients diagnosed with primary hypothyroidism and 50 patients with hyperthyroidism and 50 healthy subjects as controls. Patients recently diagnosed or previously diagnosed with hypothyroidism and untreated with LT4 replacement for at least 6 months included in the study immediately started Levothyroxine replacement therapy. Patients recently diagnosed with hyperthyroidism were included in the study before being given appropriate treatment. Patients under treatment for hypo and hyperthyroidism, type 1 and type 2 DM, chronic renal failure, chronic liver disease, chronic inflammatory diseases, and cancer were excluded from the study. Normoglycemic healthy volunteers with TSH levels between 0.4 and 4.0 mIU/l were included in the control group.

According to the ATA/AACE 2012 guideline, hypothyroidism was diagnosed as an upper limit of thyroid-stimulating hormone (TSH) ≥ 4.0 mIU/L with normal or decreased free T4 (fT4). Hyperthyroidism was diagnosed as TSH < 0.4 mIU/l with normal or increased fT4 and fT3 levels (26).

The study was conducted in agreement with the Declaration of Helsinki. The ethics committee approved the study protocol of Marmara University School of Medicine (number: 09.2011.0026). Subjects were included in the study after their informed consent was obtained. This work was supported by Marmara University Scientific Research Projects Coordination Unit under grant number SAG-A-090512-0129.

### *Clinical and laboratory assessment*

Clinical information, demographic parameters (e.g., age and gender), medication history duration and the etiology of the disease were recorded. Standing height was measured to the closest 0.5 cm using a stadiometer. Bodyweight was measured using a digital electronic scale. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters.

Serum TSH and fT4 levels were measured using an electrochemiluminescence immunoassay and Modular Analytics E170 immunoassay autoanalyzer (Roche, Mannheim, Germany). Serum creatinine and lipid levels were measured with an autoanalyzer. Glucose was measured from the serum samples following the spectrophotometric enzymatic method. Glycated hemoglobin (HbA1c) was measured using the high-performance liquid chromatography method. Thyroid autoantibodies (anti-thyroglobulin antibody and anti-thyroid peroxidase antibody) were measured by the chemiluminescence immunoassay method.

Serum CML was measured using the enzyme-linked immunosorbent assay (ELISA) method. The value range for the E1413Hu CML kit (Bioassay Technology Laboratory, Shanghai, China) was 20–3,000 ng/ml. Its sensitivity was 10.03 ng/ml. The intra-study variability coefficient for this concentration was 8%, and the inter-study variability coefficient was 10%. Serum sRAGE levels were also measured using the ELISA method. The intra-study variability coefficient for this concentration was 8%, and the inter-study variability coefficient was 12%.

### *Skin autofluorescence evaluation*

SAF measurements were done according to published protocols (13). AGE levels were measured from the forearm approximately 10 cm below the elbow fold using an AGE Reader™ (DiagnOptics Technologies, Groningen, The Netherlands) at room temperature in a semi-dark room. In short, the AGE reader brightens an approximately 4-$cm^2$ area of the skin's surface at a wavelength between 300 and 420 nm (peak intensity at ~ 370 nm), and the light emitted

and reflected from the skin is measured with an internal spectrometer in the range of 300–600 nm. SAF was calculated as the ratio between the average emitted light intensity (420–600 nm) and the average excited light intensity (300–420 nm) multiplied by 100 and presented as arbitrary units. The SAF measurement was carried out three times, and the mean of the values was accepted as the target value.

### *Statistical analysis*

Continuous variables were summarized using descriptive statistics presented as means and standard deviations (SDs). Categorical variables were summarized using counts and percentages. The Kolmogorov–Smirnov test assessed the normality of data distribution. The nonparametric analysis of variance test was used to compare the three groups, and Spearman's rank test was used for the correlation analysis. The results were evaluated at a 95% confidence interval, and a p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS program.

## RESULTS

The demographic characteristics and laboratory results for the hypothyroid, hyperthyroid, and control groups are shown in Table 1. Age, BMI values, and gender distribution were similar between the three groups.

In the hypothyroid group, 83 cases were diagnosed as chronic autoimmune thyroiditis-induced hypothyroidism, and 20 had total thyroidectomy-induced hypothyroidism due to multinodular goiter. Twenty-one of the hypothyroid cases were prescribed levothyroxine replacement therapy, 18 of them stopped the levothyroxine according to their will, and 3 of them were not aware of the lifelong treatment. The duration of the disease was 23±12 months in previously treated hypothyroid patients.

In the etiology of hyperthyroidism, 26 cases were Grave's disease, and 24 were toxic multinodular goiter/adenoma.

SAF, serum CML, and sRAGE levels of the participants are shown in Table 1. The SAF

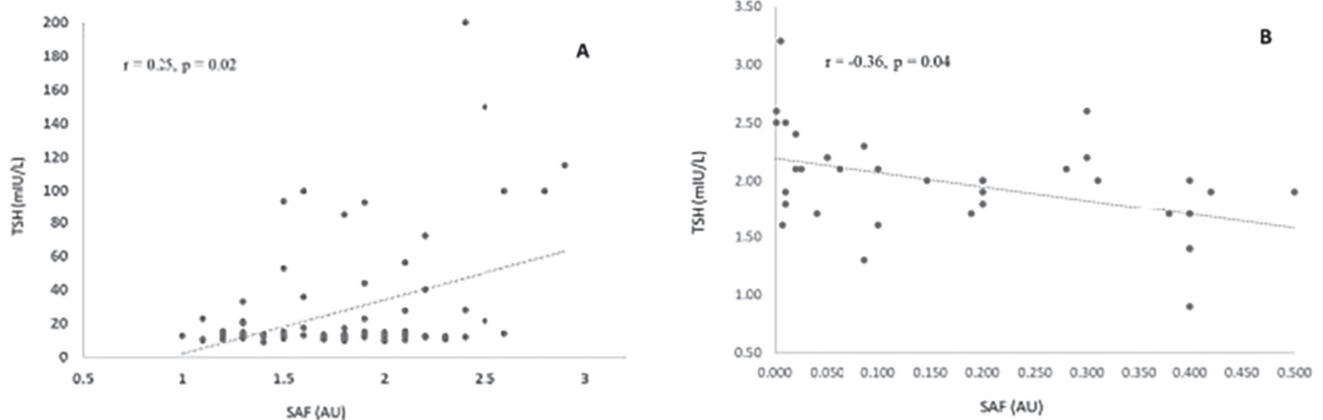


**Figure 1.** Correlation analysis between skin autofluorescence measurements and serum TSH levels in hypothyroid (A) and hyperthyperthyod Group (B).

**Table 1.** Demographic and laboratory characteristics and serum CML, sRAGE, and SAF measurements of the study groups

| | Hypothyroidism (n:103) | Hyperthyroidism (n:50) | Control (n:50) | *p* |
|---|---|---|---|---|
| **Age (year)** | 42±13 | 47±10 | 46±13 | 0.8 |
| **Gender (F/M)** | 96/7 | 48/2 | 48/2 | 0.8 |
| **BMI (kg/m$^2$)** | 27.9±6.4 | 28.9±5.1 | 29.4±5.2 | 0.8 |
| **TSH (mIU/L)** | 20.9±10 | 0.08±0.1 | 2.2±1.0 | 0.001 |
| **fT4 (ng/dL)** | 0.67±0.3 | 1.4±0.3 | 0.9±1.0 | 0.01 |
| **Fasting glucose (mg/dL)** | 94±8 | 100±16 | 97±16 | 0.7 |
| **HbA1c (%)** | 5.4±0.8 | 5.6±0.6 | 5.2±0.4 | 0.6 |
| **CML (ng/mL)** | 8.2±2.8 | 10.2±2.0 | 8.0±3.3 | 0.01 |
| **sRAGE (ng/mL)** | 327±225 | 238±37 | 291±98 | 0.6 |
| **SAF (AU)** | 1.82±0.04 | 1.80±0.40 | 1.63±0.30 | 0.04 |

BMI: Body mass index, CML: Nepsilon-(carboxymethyl)lysine, sRAGE:serum soluble receptors for AGEs, SAF: Skin autofluorescence.

measurements for the hyper- and hypothyroid groups were significantly higher than those of the control group (p = 0.04). Serum CML levels were significantly higher in the hyperthyroid group compared to the hypothyroid and control groups (p = 0.01), while sRAGE levels were similar between the three groups (p=0.62).

There were no statistically significant differences in SAF(p =0.61), CML(p=0.72), or sRAGE (p=0.67) values between Hashimoto's disease and total thyroidectomy patients in the hypothyroid group. Similarly, there were no statistically significant differences in SAF(p=0.78), CML(p=0.8), or sRAGE(p=0.73) values between Graves disease and toxic multinodular goiter/adenoma in the hyperthyroid group.

***Correlation analysis***

There was a significant positive correlation between SAF measurements and HbA1c values in the whole group (r = 0.30, p = 0.03). There was a significant positive correlation between serum TSH level and SAF measurements in the hypothyroid group (r = 0.25, p = 0.02). Conversely, a significant negative correlation was observed between serum TSH levels and SAF measurements in the hyperthyroid group (r = -0.36, p = 0.04) (Fig. 1). All three groups had no correlations between SAF measurements, serum CML, and sRAGE levels.

## DISCUSSION

In this study, SAF measurements were higher for the hypo- and hyperthyroidism groups than the control group. There was a positive correlation between serum TSH levels and SAF measurements in the hypothyroid group; conversely, a negative correlation was observed between serum TSH levels and SAF measurements in the hyperthyroid group.

Although serum CML was higher in the hyperthyroidism group than in the hypothyroidism and control groups, serum sRAGE levels were similar.

According to a recent literature review, this is the first study to evaluate skin-accumulated AGEs and serum AGE levels in thyroid hormone dysfunction.

Excess and lower states of thyroid hormones have many effects on the circulatory system. Prior studies have shown an increased risk of cardiovascular disease and mortality with thyroid dysfunction, particularly hypothyroidism (27). Endothelial dysfunction increased atherosclerosis, and coagulation system disorders are associated with hypothyroidism (28). The early atherosclerosis marker of carotid intima-media thickness is increased in overt and subclinical hypothyroidism. Hyperthyroidism is mainly related to atrial fibrillation and increased oxidative stress (30).

The validity of SAF as a tissue biomarker of cardiovascular and all-cause mortality risk in patients with DM, cardiovascular disease, and renal disease has been proven in numerous studies (14-17).

AGE formation is associated with the development of OS (29). Interaction of AGEs with specific AGE receptors called RAGEs increases intracellular reactive oxygen species (ROS). OS can be defined as an imbalance between the production of pro-oxidant substances and antioxidant defenses. ROS is one of the essential pro-oxidants. Both hyperthyroidism and hypothyroidism are associated with OS (30). However, how OS is developed differs between these two clinical situations. There is low availability of antioxidants in hypothyroidism, whereas, in hyperthyroidism, there is an increase in ROS production. Our study shows that SAF is increased in hypo- and hyperthyroidism, which provides another probable explanation for increased OS in thyroid dysfunction.

Increased SAF levels in our group cannot be attributed to hyperglycemia and abnormal kidney function, as patients were normoglycemic and had normal kidney function.

Although skin AGE accumulation increased in hypothyroid and hyperthyroid patients, serum CML levels were elevated in hyperthyroid patients compared to hypothyroid patients and the control group. Increased ROS production in hyperthyroidism may explain high CML levels in hyperthyroidism and needs to be clarified with further prospective studies.

Thyroid hormones have significant effects on both glucose and lipid metabolism. They stimulate gluconeogenesis and glycogenolysis, reduce insulin sensitivity, and increase insulin metabolism; thus, as glucose intolerance and peripheral insulin resistance occur, diabetes worsens (31). Thyroid hormones promote albumin catabolism, so thyroid dysfunction may also alter glycated albumin levels. In our study, glucose and HbA1c levels were similar in hypo- and hyperthyroid groups. Although hyperglycemia triggers AGE formation, it is not always a necessary condition. In many clinical presentations, AGEs can occur due to OS, like inflammatory diseases without hyperglycemia.

This study shows that skin AGEs increase in thyroid dysfunction and serum CML increases in

hyperthyroid patients without hyperglycemia.

The limitations of this study are the lack of data on disease duration, oxidative stress parameters, and other serum AGEs such as pentosidine. Due to the study's cross-sectional nature, we cannot determine a causal relation between AGEs and thyroid hormone status.

**In conclusion,** the results of this study indicate that increased serum and skin AGE levels can be one of the possible mechanisms between increased cardiovascular risk and a hypo-hyperthyroid state.

Further prospective clinical studies are needed to evaluate the relationship between AGEs and cardiovascular risk and the effects of treatment for hypo and hyperthyroidism.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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**Ethics approval**

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Marmara University (number: 09.2011.0026).

Informed consent was obtained from all individual participants included in the study.

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