Endocrine (2015) 48:916–923
DOI 10.1007/s12020-014-0399-3

ORIGINAL ARTICLE

Assesment of oxidative status and its association with thyroid autoantibodies in patients with euthyroid autoimmune thyroiditis

Husniye Baser · Ummugulsum Can · Salih Baser · Fatma Humeyra Yerlikaya · Uysaler Aslan · Bahauddin Taha Hidayetoglu

Received: 24 June 2014 / Accepted: 14 August 2014 / Published online: 23 August 2014

Abstract Oxidative stress results from either overproduction of free radicals or insufficiency of several anti-oxidant defense systems. It leads to oxidation of main cellular macromolecules and a resultant molecular dysfunction. Thyroid hormones regulate oxidative metabolism and, thus, play a role in free radical production. Studies evaluating oxidative stress in patients with hypothyroidism and hyperthyroidism have been encountered in recent years; however, oxidative status in patients with euthyroid autoimmune thyroiditis (AIT) was not investigated previously. Thirty-five subjects with euthyroid AIT and 35 healthy controls were enrolled in the study. Serum oxidative status was determined by the measurement of total anti-oxidant status (TAS), total oxidant status (TOS), ischemia-modified albumin (IMA), and oxidized-low density lipoprotein (ox-LDL) levels. Serum TAS levels were significantly lower ($p < 0.001$), while serum TOS levels and IMA levels were significantly higher ($p < 0.001$ and $p = 0.020$, respectively) in patients compared to controls. In both groups, ox-LDL levels were similar ($p = 0.608$). Serum TAS levels were negatively correlated with anti-thyroid peroxidase and anti-thyroglobulin (anti-TG) levels (rho $= -0.415$, $p = 0.001$ and rho $= -0.484$, $p < 0.001$, respectively). Serum TOS was positively correlated with anti-TG levels (rho $= 0.547$, $p < 0.001$). Further, TAS was positively correlated with free T4 levels ($r = 0.279$, $p = 0.043$). No correlation was observed between thyrotropin, free T3 levels, and TOS and TAS levels. These results suggest that oxidants are increased, and anti-oxidants are decreased in patients with euthyroid AIT, and oxidative/anti-oxidative balance is shifted to the oxidative side. Increased oxidative stress might have a role in thyroid autoimmunity.

Keywords Euthyroid · Autoimmune thyroiditis · Oxidative stress

H. Baser (✉)
Department of Endocrinology and Metabolism, Konya Education and Research Hospital, Meram, Konya, Turkey
e-mail: drhusniyebaser@yahoo.com.tr

U. Can
Department of Biochemistry, Konya Education and Research Hospital, Konya, Turkey

S. Baser · U. Aslan · B. T. Hidayetoglu
Department of Internal Medicine, Konya Education and Research Hospital, Konya, Turkey

F. H. Yerlikaya
Department of Biochemistry, Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey

Introduction

Thyroid hormones are one of the most important factors influencing the basal metabolic rate during normal physiological states. They alter oxygen consumption in mitochondria which is the main production site of free radicals [1]. Oxygen-free radicals are significantly influential on the pathogenesis of tissue damage of several pathologic conditions including hypothyroidism and hyperthyroidism [2–4]. Reactive oxygen species (ROS) are highly reactive molecules that, when present in excess, overwhelm the protective systems, and result in cell damage and lipid peroxidation [2, 5]. ROS are formed in oxidative processes that normally occur at relatively low levels in all cells and tissues [2]. In normal situations, a number of anti-oxidant mechanisms serve to control this production [6]. In contrast, high doses and/or inadequate removal of ROS result

in oxidative stress leading to severe metabolic dysfunctions and damage to biological macromolecules [7]. The measurements of total anti-oxidant status (TAS) and total oxidant status (TOS) can be used for the prediction of oxidative status [8]. Especially the measurement of TAS reflects the overall anti-oxidant state in an organism.

Ischemia-modified albumin (IMA) is a promising biomarker used in order to evaluate patients with ischemic events. Previous studies demonstrated that IMA is not only specific for cardiac ischemia, but is also elevated in various diseases [9–11]. The production of IMA seems to be associated with the production of ROS modifying the metal-binding sites of albumin [12, 13]. IMA is considered a non-specific biomarker in the evaluation of oxidative stress status or atherosclerosis burden [10, 11]. Oxidized-low density lipoprotein (ox-LDL) emerges from binding of low density lipoprotein cholesterol (LDL-C) with unsaturated fatty acids. Ox-LDL is involved in the pathogenesis of atherosclerosis, because ox-LDL infiltrates macrophages and converts them into foam cells [14, 15].

In the literature, studies investigating oxidative status in patients with hyperthyroidism and hypothyroidism by the measurements of TAS, TOS, IMA, and ox-LDL are present [16–21]. To the best of our knowledge, no studies investigated the oxidative status in patients with euthyroid autoimmune thyroiditis (AIT). Therefore, we aimed to evaluate oxidative status by TAS, TOS, IMA, and ox-LDL in patients with euthyroid AIT.

Materials and methods

Subjects

This study included 35 patients with euthyroid AIT. The patient group was compared with 35 healthy controls, who were matched for age, sex, and body mass index (BMI). Patients with a history of thyroid dysfunction, diabetes mellitus, hypertension, liver or pulmonary diseases, malignancy, renal, coronary heart, or rheumatologic diseases, and those using drugs affecting oxidative status, taking a thyroid medication and cigarette smokers constituted our exclusion criteria. In addition, any subject with an abnormal free T4 (FT4) concentration or thyrotropin (TSH) concentration was excluded. The diagnosis of AIT was based on positive anti-thyroid antibodies and thyroid ultrasonography. Patients with positive anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies at the same time with a moderate to severe parenchymal heterogeneity by thyroid ultrasonography were described to have AIT and included to the study. The control group consisted of healthy individuals with no history of thyroid disease. The study was approved by the ethical board of the institution, and informed consent was obtained from all participants.

Blood samples

Blood samples were drawn from the antecubital vein, after an overnight fasting. Venous blood samples were centrifuged at 3,000 rpm for 10 min, and samples were stored at −80 °C until analysis. TSH, FT4, free T3 (FT3), anti-TPO, and anti-TG values were measured with chemiluminescence method (cobas e 601 hormone auto-analyser Roche Diagnostic System). The reference ranges were 0.27–4.20 μIU/mL for TSH, 0.85–1.7 ng/dL for FT4, 2.0–4.4 pg/mL for FT3, 0–35 IU/mL for anti-TPO, and 0–115 IU/mL for anti-TG. Serum albumin, triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C), and LDL-C levels were measured using commercially available kits based on routine methods on Architect C 8000 System (Abbott Laboratories, Abbott Park, Illinois, USA).

Measurement of TAS

Serum TAS was determined using an automated measurement method based on the bleaching of characteristic color of a more stable 2,2′-azino-bis (3-ethylbenz-thiazoline 6-sulfonic acid) (ABTS) radical cation by anti-oxidants [8]. In this measurement, the results are expressed in mmol Trolox equivalents/L(mmol Trolox equiv./L).

Measurement of TOS

Serum TOS was defined via a novel automated measurement method [22]. Oxidants that are present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide (H_2O_2), and the results are expressed in terms of micromolar H_2O_2 equivalents per liter (μmol H_2O_2 equiv./L).

Measurement of IMA

IMA level was measured using a colorimetric assay developed by Bar-Or et al. [23] and based on the measurement of unbound cobalt after incubation with patients' sera. Increased amounts of IMA result in less cobalt binding and more residual unbound cobalt available for complex with a chromogen [dithiothreitol (DTT)], which can be measured photometrically. The procedure

was performed as follows: 50 μL of 0.1 % cobalt chloride was added to 200 μL of serum, gently mixed, and held for 10 min for adequate cobalt-albumin binding. Fifty microliters of DTT, at a concentration of 1.5 mg/mL, was added as a colorizing agent, and the reaction was stopped 2 min later by adding 1.0 mL of 0.9 % NaCl. The colored product was measured at 470 nm, compared with a serum-cobalt blank without DTT and reported in absorbance units (ABSU).

Adjusted IMA was calculated as (individual serum albumin concentration/median serum albumin concentration of the population) × IMA ABSU value. This formula was applied to correct IMA values for serum albumin. The median serum albumin concentration of each group was used separately [24].

Measurement of ox-LDL levels

Analysis of ox-LDL was performed on serum samples through Mercodia ox-LDL ELISA kit (Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden) in accordance with the manufacturer's guidelines. In the measurement, absorbance was measured at 450 nm on an ELx800 Absorbance Microplate Reader (Biotek, Winooski, VT, USA). This assay employs a quantitative sandwich enzyme immunoassay technique measuring ox-LDL. The resulting concentration values are reported in U/L. Although the manufacturer advices that each laboratory establishes its own expected range of values, it also declares that mean value of ox-LDL was found to be 61 U/L (26–117) in 149 ambulatory, randomly selected individuals.

Statistical analysis

All statistical analyses were performed with SPSS 15.0 (SPSS Inc., Il., USA) statistical soft-ware. The Kolmogorov–Smirnov test was used for the compliance with the normal distribution. All parameters except anti-TPO and anti-TG were within the normal distribution ranges. The comparisons between groups were performed by the student's *t* test for parametric variables, and the Mann–Whitney U test for non-parametric variables. Descriptive analyses were presented using mean ± standard deviation for normally distributed variables, median and range (min–max) for non-normally distributed variables. The Chi-square test was used to investigate the differences between groups regarding the categorical variables. The Pearson's and Spearman's correlation analyses were performed in order to document possible associations between parametric and non-parametric variables, respectively. The correlation analysis was made including all subjects (patients and controls). A p value less than 0.05 was accepted as statistically significant.

Table 1 Demographic and biochemical data of patients with euthyroid autoimmune thyroiditis and controls

	Patients ($n = 35$)	Controls ($n = 35$)	p
Age (yrs)	36.20 ± 9.45	39.66 ± 8.69	0.131
Female/Male	27/8	26/9	0.780*
BMI (kg/m^2)	29.75 ± 3.79	28.38 ± 4.03	0.149
Fasting plasma glucose (mg/dL)	90.57 ± 6.30	92.08 ± 12.37	0.562
Total cholesterol (mg/dL)	187.68 ± 39.49	184.00 ± 29.10	0.726
LDL-C (mg/dL)	113.97 ± 30.22	115.15 ± 27.25	0.894
HDL-C (mg/dL)	48.68 ± 8.07	44.75 ± 8.67	0.136
Triglycerides (mg/dL)	123.90 ± 73.50	122.04 ± 47.72	0.921
TSH (μIU/mL)	2.34 ± 1.24	1.86 ± 0.71	0.074
FT3 (pg/mL)	3.35 ± 0.36	3.54 ± 0.33	0.076
FT4 (ng/dL)	1.17 ± 0.22	1.25 ± 0.26	0.219
Anti-TPO (IU/mL)	169.35 (7.09–1,000.00)	11.80 (10.00–30.50)	<0.001
Anti-TG (IU/mL)	177.40 (15.59–1,355.00)	20.00 (20.00–20.70)	<0.001
TAS (mmol Trolox equiv./L)	1.28 ± 0.19	1.41 ± 0.13	<0.001
TOS (μmol H_2O_2 equiv./L)	5.30 ± 2.18	3.03 ± 1.14	<0.001
IMA (ABSU)	0.44 ± 0.18	0.34 ± 0.15	0.020
Ox-LDL (U/L)	75.74 ± 20.36	78.57 ± 15.82	0.608

BMI body mass index, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *TSH* thyrotropin, *FT3* free T3, *FT4* free T4, *anti-TPO* anti-thyroid peroxidase, *anti-TG* anti-thyroglobulin, *TAS* total anti-oxidant status, *TOS* total oxidant status, *IMA* ischemia-modified albumin, *ox-LDL* oxidized-LDL, * χ^2

Results

There were 35 patients (8 male, 27 female) with euthyroid AIT, with a mean age of 36.2 ± 9.4 years. Healthy control group included 9 male and 26 female patients, and mean age was 39.6 ± 8.6 years. There were no significant differences between patients and controls in respect to age, gender, and BMI (for all parameters, $p > 0.05$). Demographic and laboratory findings of patients and controls were presented in Table 1. No significant difference was found between groups regarding to fasting plasma glucose, total cholesterol, LDL-C, HDL-C, and triglycerides ($p > 0.05$). Serum TSH, FT3, and FT4 levels ($p = 0.074$, $p = 0.076$ and $p = 0.219$, respectively) were similar in patients and control group. Anti-TPO and anti-TG levels

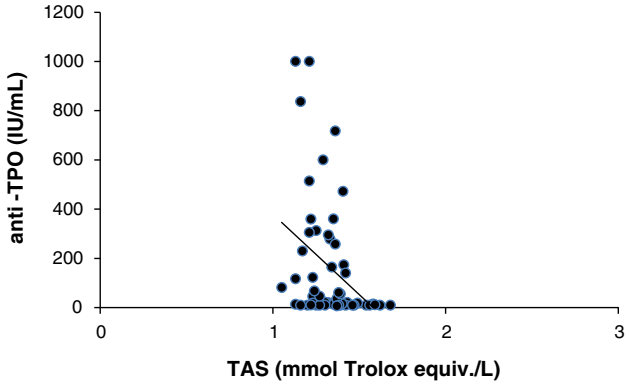

Fig. 1 The correlation between anti-TPO and TAS levels

Table 2 Association between oxidative stress markers and thyroid autoantibodies

	Anti-TPO (rho, *p*)		Anti-TG (rho, *p*)	
TAS (mmol Trolox equiv./L)	−0.415	0.001	−0.484	<0.001
TOS (μmol H_2O_2 equiv./L)	0.238	0.078	0.547	<0.001
IMA (ABSU)	0.133	0.358	−0.089	0.547
Ox-LDL (U/L)	0.111	0.473	0.017	0.916

rho Spearman's correlation coefficient, *anti-TPO* anti-thyroid peroxidase, *anti-TG* anti-thyroglobulin, *TAS* total anti-oxidant status, *TOS* total oxidant status, *IMA* ischemia-modified albumin, *ox-LDL* oxidized-LDL

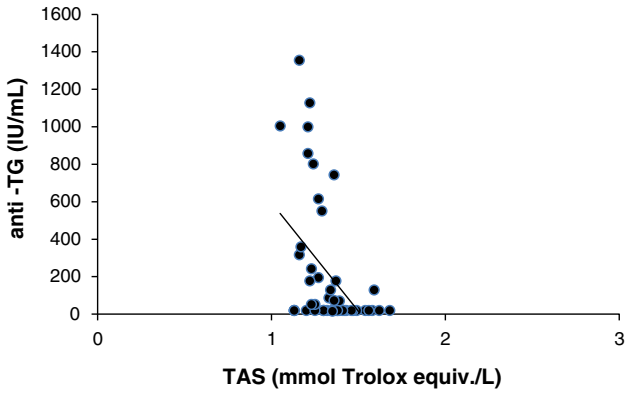

Fig. 2 The correlation between anti-TG and TAS levels

were significantly higher in patients compared with those in controls ($p < 0.001$ for each).

IMA and TOS levels were higher in patients compared to controls ($p = 0.020$ and $p < 0.001$, respectively). In addition, patients had lower TAS levels compared to controls ($p < 0.001$), while ox-LDL levels were similar in two groups ($p = 0.608$).

A negative correlation was observed between anti-TPO and TAS levels (rho = −0.415, $p = 0.001$) (Fig. 1)

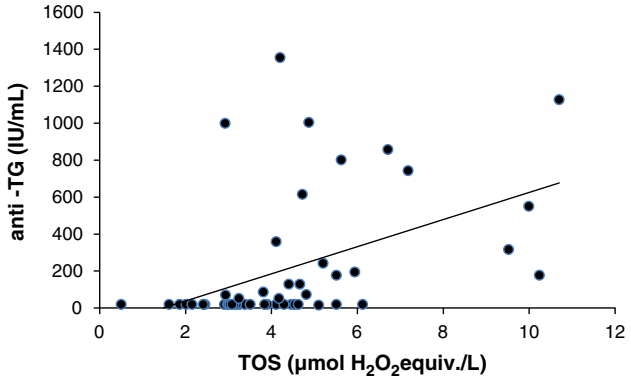

Fig. 3 The correlation between anti-TG and TOS levels

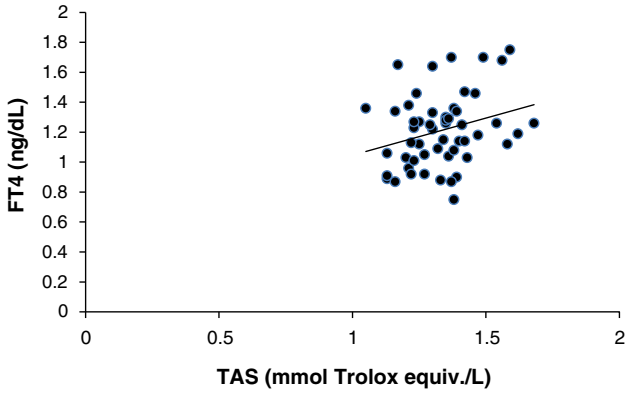

Fig. 4 The correlation between FT4 and TAS levels

although no significant correlation was found between anti-TPO, and TOS, IMA, and ox-LDL levels (Table 2).

Anti-TG was correlated negatively with TAS levels (rho = −0.484, $p < 0.001$) (Fig. 2) and positively correlated with TOS levels (rho = 0.547, $p < 0.001$) (Fig. 3). There was no significant correlation between anti-TG levels, and IMA and ox-LDL levels (Table 2).

No significant correlation was found between TSH, FT3 levels, and TAS, TOS, ox-LDL, and IMA levels (for all parameters, $p > 0.05$). FT4 was positively correlated with TAS ($r = 0.279$, $p = 0.043$) (Fig. 4), and no significant correlation was found between FT4 and TOS, IMA, and ox-LDL levels (for all parameters, $p > 0.05$). In addition, IMA and ox-LDL levels were not significantly correlated with TAS and TOS levels (for all parameters, $p > 0.05$).

Discussion

In the present study, we assessed the oxidative status of patients with euthyroid AIT using TAS, TOS, IMA, and

ox-LDL, and observed that these patients had decreased TAS levels, along with increased TOS and IMA levels. However, there were no differences in ox-LDL levels between groups. Both the increase in oxidants and decrease in anti-oxidants were responsible for increased oxidative stress in our study group. To the best of our knowledge, our study is the first to investigate the oxidative status in euthyroid AIT subjects by TAS, TOS, IMA, and ox-LDL measurements.

Thyroid hormones are associated with oxidative and anti-oxidative status of an organism, thus playing an important role in the production of ROS and free radicals [25, 26]. Vice versa, ROS and free radicals are responsible for physiological and pathological processes, and so lead to redox imbalance in thyroid [27, 28]. Anti-oxidative defense pathways of an organism play a crucial role in reducing the increased levels of free radicals generated by thyroid gland dysfunction [29]. In hyperthyroidism, augmented production of ROS is observed as a result of increased basal metabolic rate, which causes increased oxidative stress in such patients in the presence of inadequate anti-oxidative defense [30]. In a recent study, where total anti-oxidant capacity (TAC) and TOS were studied in patients with hyperthyroidism, serum TAC was found to be significantly lower, while serum TOS levels were significantly higher in hyperthyroid patients. Also, serum TAC and TOS levels were observed to be correlated with TSH, FT3, and FT4 levels in these patients [17]. There is evidence that ROS might contribute to the pathogenesis of Graves ophthalmopathy [31]. In a study, oxidative stress profile was investigated in patients with Graves ophthalmopathy before and after normalization of thyroid hormones. Although the values of ROS decreased and levels of anti-oxidants got corrected significantly after anti-thyroid treatment, oxidative stress levels remained significantly elevated as compared to normal persons [32].

Although some studies report that overt hypothyroidism is expected to decrease the free radical generation because of an associated lower metabolic rate [33, 34], others state an increased oxidative stress in overt hypothyroidism [3, 4, 16]. Recently, Reddy et al. showed that hypothyroid patients had a deficient anti-oxidant defense system in the form of decreased activity of superoxide dismutase (SOD) and decreased levels of ferric reducing ability of plasma and reduced glutathione [35]. In addition, thyroidectomy or thyroparathyroidectomy were shown to be associated with oxidative stress despite the application of replacement therapies [36].

Initially emerging as a marker of ischemia, IMA is thought to be beneficial in the identification of acute coronary syndromes. However, because it is not tissue specific, IMA is also elevated in individuals undergoing oxidative stress other than cardiac ischemia. IMA may be a valuable biomarker for oxidative stress in patients with overt thyroid dysfunction. Ma et al. reported that IMA levels were increased in patients with thyroid dysfunction, particularly in overt hypothyroidism, and that IMA levels were significantly negatively correlated with FT3 and FT4 levels. In the same study, IMA levels were found to be positively correlated with anti-TPO levels [19]. In contrary, recently, in a study including 34 hypothyroid, 27 hyperthyroid, and 27 euthyroid subjects, IMA was significantly lower in hypothyroid and higher in hyperthyroid group compared to euthyroid group [37]. However, Ersoy et al. stated that serum IMA levels did not differ among patients with overt or subclinical hypothyroidism [18]. In the literature, no studies evaluating IMA levels in euthyroid AIT patients were encountered. In our study, IMA levels were found to be higher in patients with euthyroid AIT, but no significant association was present between IMA levels, and TSH, FT4, FT3, and thyroid autoantibodies. Higher levels of IMA in patients with euthyroid AIT may indicate co-existing cardiovascular risk factors and oxidative stress.

An increase in oxidative stress and a resultant lipid peroxidation may follow the tissue hypoxia [38, 39]. Hypothyroidism and hyperthyroidism both affect the oxidation of LDL, and the oxidation of LDL may contribute to the increased risk of atherosclerosis [21]. Evidence suggests that when compared to euthyroid individuals, LDL-C levels are lower in patients with overt hyperthyroidism and higher in those with overt hypothyroidism. In contrast, the oxidization of LDL-C was reported to be increased in overt hyperthyroidism [4, 40, 41]. This might be due to the increased free radical production in mitochondria leading to changes in the anti-oxidant defense mechanism [33]. Additionally, several studies also report higher oxidization rates in hypothyroidism [4, 40, 41]. Such a condition might be the result of LDL-C acting as a pro-oxidant factor, and lower T4 concentrations induce a higher oxidation rate of LDL-C [4, 42]. In our study, no difference was found between patients with euthyroid AIT and controls in terms of LDL-C and ox-LDL levels. It is a known entity that LDL-C is the main factor for increase in ox-LDL levels. The fact that no difference was observed between the groups in our study as to ox-LDL levels may be attributed to similar LDL-C levels found in two groups.

Excessive production of ROS and altered redox state are considered as one of the pathogenic mechanisms underlying systemic autoimmune response [43]. Increased ROS may cause oxidative modification of lipid, protein, carbohydrate, and DNA which might serve as neoantigens leading loss of self tolerance. Oxidative modification of proteins has been shown to induce pathogenic antibodies in a variety of autoimmune diseases [43]. Several biomarkers of oxidative stress such as malondialdehyde-modified proteins (MDA), F2-isoprostane (8-isoPGF2), nitric oxide,

and 4-hydroxy-2-nonenal were found to increase in patients with systemic lupus erythematosus, and some were found to be associated with disease severity and activity [44]. In addition, diminished levels of intracellular reduced glutathione and SOD-1 activity which have anti-oxidant capacity were reported in these patients [44]. Also, in patients with vitiligo, increased intracellular production of ROS and decreased anti-oxidant status were observed, and oxidative stress was considered to be one of the pathogenic mechanisms [45]. In addition, the ratio of reduced to oxidized glutathione which is inversely proportional to degree of oxidative stress was found to be higher in acute immune thrombocytopenia (ITP) patients compared to chronic ITP patients suggesting the role of oxidative stress at the beginning of the autoimmune process [46]. Type 1 diabetes mellitus is another autoimmune disease with a documented role of oxidative stress in the disease onset [47].

In our study, we found increased oxidative stres and a shift of oxidative/anti-oxidative balance to the oxidative side in patients with autoimmune thyroiditis independent from thyroid functions. In addition, a significant association was observed between thyroid autoantibodies and oxidative stress parameters (TAS and TOS). This provides evidence for possible role of oxidative stress in the pathogenesis of autoimmune thyroid diseases. In a recent trial, a substantial reduction in serum glutathione status and a relation between glutathione and anti-TPO antibodies were demonstrated in 44 female patients with newly diagnosed Hashimoto thyroiditis [48]. The authors concluded that glutathione diminution might be a hallmark leading to oxidative stress and the development of immunological intolerance in Hashimoto thyroiditis. However, in contrary to our study, in that study, serum TSH was significantly higher in patients compared to control subjects, which may constitute a possible confounding factor effecting oxidative status.

Oxidative stress seems to have a role in the aging of the endocrine system and in the pathogenesis of several endocrine diseases including autoimmune thyroid diseases [49, 50]. It was shown that exposure to high concentrations of H_2O_2 induces thyroglobulin (TG) fragmentation in cultures of human thyroid cells [51]. During aging, prolonged exposure of thyrocytes to H_2O_2 and/or impairment of the anti-oxidant system might be the triggering factor leading to morphological and functional damage and alterations in the antigenicity of TG and thyroid peroxidase [51]. Additionally, accumulation of ROS in the thyroid gland with age causes increased expression of intercellular adhesion molecule 1 (ICAM-1) on thyrocytes which have a key role in the onset of inflammatory responses [52]. Despite these data, it is not possible to answer yet whether increased oxidative stress is the result or part of the cause of chronic inflammation and autoimmunity.

The method of oxidative stress measurement is very important. At present, there is no single method that can accurately measure the oxidative stress or its subsequent damage [53]. Criteria for an ideal biomarker of oxidative damage were defined by Halliwell et al. [54]. The authors have concluded that F2-isoprostanes and other isoprostanes in tissues and body fluids (lipid peroxidation), 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine (damage to DNA and the DNA precursor pool), and comet assay with cleavage enzymes (DNA damage) were reliable methods to determine oxidative stress. One of the methods to determine anti-oxidant status is to measure individual anti-oxidants (e.g., ascorbate, alpha tocopherol, and urate) in blood, plasma, or tissue homogenates. However, this approach may not reflect the interplay between different anti-oxidants, and the effect of presently unknown anti-oxidant substances will be omitted by this method. Measurement of TAC or activity provides detection of cumulative action of all the anti-oxidants present in plasma and body fluids.

There are colorimetric, fluorescence, and chemiluminescence methods developed to measure ROS and determine TAS [55, 56]. However, there is not yet an accepted reference method and each has their own advantages and disadvantages such as requirement of sophisticated techniques, invalid results, inappropriate for automated analyzers and instability of assay reagents. The most commonly used colorimetric measurement method for TAS is ABTS based methods. In our study, we determined TAS using an automated measurement method based on the bleaching of characteristic color of a more stable ABTS radical cation by anti-oxidants [8]. This method was shown to be significantly correlated with the other TAC measurement methods. For determination of TOS, we used a novel method of which the main components are H_2O_2 and lipid hydroperoxide [22].

There are some limitations in our study. First, our sample size was small. Second, we used TAS, TOS, IMA, and ox-LDL measurements for evaluation of oxidative status. We did not measure F2-isoprostanes, other isoprostanes, or hydroxy-2′-deoxyguanosine levels which are more widely used biomarkers to determine oxidative damage. Also, we did not measure urate levels which are widely available anti-oxidant marker. However, the methods we used for measurement of TAS and TOS are established to be easy, rapid, stable, reliable, inexpensive, sensitive, and correlated with the other TAC measurement methods. In addition, they were used to show oxidative status previously in various studies [17, 57].

In conclusion, the oxidative/anti-oxidative balance is in favor of the oxidative side in patients with euthyroid AIT. In addition, our results suggest that increased oxidative stress is likely to be associated with thyroid autoimmunity.

So, further studies including larger sample size and evaluating multiple parameters of oxidative status are required to investigate possible role of oxidative stress on autoimmune thyroid diseases.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standards This study approved by the local ethical committee (Approval date and number- 26.12.2012/2012-75).

References

1. L. Oziol, P. Faure, N. Bertrand, P. Chomard, Inhibition of in vitro macrophage-induced low density lipoprotein oxidation by thyroid compounds. J. Endocrinol. **177**(1), 137–146 (2003)
2. B. Halliwell, Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet **344**(8924), 721–724 (1994)
3. L. Dumitriu, R. Bartoc, H. Ursu, M. Purice, V. Lonescu, Significance of high levels of serum malonyl dialdehyde (MDA) and ceruloplasmin (CP) in hyper- and hypothyroidism. Endocrinologie **26**(1), 35–38 (1988)
4. F. Costantini, S.D. Pierdomenico, D. De Cesare, P. De Remigis, T. Bucciarelli, G. Bittolo-Bon, G. Cazzolato, G. Nubile, M.T. Guanano, S. Sensi, F. Cuccurullo, A. Mezzetti, Effect of thyroid function on LDL oxidation. Arterioscler. Thromb. Vasc. Biol. **18**(5), 732–737 (1998)
5. J.M. Gutteridge, Free radicals in disease processes: a compilation of cause and consequence. Free Radic. Res. Commun. **19**(3), 141–158 (1993)
6. I. Fridovich, Superoxide anion radical (O2-), superoxide dismutases, and related matters. J. Biol. Chem. **272**(30), 18515–18517 (1997)
7. B.S. Berlett, E.R. Stadtman, Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem. **272**(33), 20313–20316 (1997)
8. O. Erel, A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem. **37**(4), 277–285 (2004)
9. S.G. Ma, W. Xu, C.L. Wei, X.J. Wu, B. Hong, Z.J. Wang, H.R. Hao, H.Q. Guo, Role of ischemia-modified albumin and total homocysteine in estimating symptomatic lacunar infarction in type 2 diabetic patients. Clin. Biochem. **44**(16), 1299–1303 (2011)
10. S. Turedi, O. Cinar, I. Yavuz, A. Mentese, A. Gunduz, S.C. Karahan, M. Topbas, E. Cevik, A.O. Yildirim, A. Uzun, U. Kaldirim, Differences in ischemia-modified albumin levels between end stage renal disease patients and the normal population. J. Nephrol. **23**(3), 335–340 (2010)
11. C.Y. Chen, W.L. Tsai, P.J. Lin, S.C. Shiesh, The value of serum ischemia-modified albumin for assessing liver function in patients with chronic liver disease. Clin. Chem. Lab. Med. **49**(11), 1817–1821 (2011)
12. D. Bar-Or, G. Curtis, N. Rao, N. Bampos, E. Lau, Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. Eur. J. Biochem. **268**(1), 42–47 (2001)
13. S. Gidenne, F. Ceppa, E. Fontan, F. Perrier, P. Burnat, Analytical performance of the albumin cobalt binding (ACB) test on the Cobas MIRA Plus analyzer. Clin. Chem. Lab. Med. **42**(4), 455–461 (2004)
14. H. Esterbauer, G. Wag, H. Puhl, Lipid peroxidation and its role in atherosclerosis. Br. Med. Bull. **49**(3), 566–576 (1993)
15. D. Steinberg, S. Parthasarathy, T.E. Carew, J.C. Khoo, J.L. Witztum, Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Eng. J. Med. **320**(14), 915–924 (1989)
16. A.N. Torun, S. Kulaksizoglu, M. Kulaksizoglu, B.O. Pamuk, E. Isbilen, N.B. Tutuncu, Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. Clin. Endocrinol. (Oxf) **70**(3), 469–474 (2009)
17. M. Aslan, N. Cosar, H. Celik, N. Aksoy, A.C. Dulger, H. Begenik, Y.U. Soyoral, M.E. Kucukoglu, S. Selek, Evaluation of oxidative status in patients with hyperthyroidism. Endocrine **40**(2), 285–289 (2011)
18. K. Ersoy, İ. Anaforoglu, E. Algün, Serum ischemic modified albumin levels might not be a marker of oxidative stress in patients with hypothyroidism. Endocrine **43**(2), 430–433 (2013)
19. S.G. Ma, L.X. Yang, F. Bai, W. Xu, B. Hong, Ischemia-modified albumin in patients with hyperthyroidism and hypothyroidism. Eur. J. Intern. Med. **23**(6), 136–140 (2012)
20. M. Lampka, R. Junik, A. Nowicka, E. Kopczyńska, T. Tyrakowski, G. Odrowaz-Sypniewska, Oxidative stress markers during a course of hyperthyroidism. Endokrynol. Pol. **57**(3), 218–222 (2006)
21. A. Oge, E. Sozmen, A.O. Karaoglu, Effect of thyroid function on LDL oxidation in hypothyroidism and hyperthyroidism. Endocr. Res. **30**(3), 481–489 (2004)
22. O. Erel, A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. **38**(12), 1103–1111 (2005)
23. D. Bar-Or, E. Lau, J.V. Winkler, A Novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia- a preliminary report. J. Emerg. Med. **19**(4), 311–315 (2000)
24. G. Lippi, M. Montagnana, G.L. Salvagno, G.C. Guidi, Standardization of ischemia-modified albumin testing: adjustment for serum albumin. Clin. Chem. Lab. Med. **45**(2), 261–262 (2007)
25. M.J. Coria, A.I. Pastán, M.S. Gimenez, Serum oxidative stress parameters of women with hypothyroidism. Acta Biomed. **80**(2), 135–139 (2009)
26. H. Erdamar, B. Cimen, H. Gülcemal, R. Saraymen, B. Yerer, H. Demirci, Increased lipid peroxidation and impaired enzymatic antioxidant defense mechanism in thyroid tissue with multinodular goiter and papillary carcinoma. Clin. Biochem. **43**(7–8), 650–654 (2010)
27. L.E. Laatikainen, M.D. Castellone, A. Hebrant, C. Hoste, M.C. Cantisani, J.P. Laurila, G. Salvatore, P. Salerno, F. Basolo, J. Näsman, J.E. Dumont, M. Santoro, M.O. Laukkanen, Extracellular superoxide dismutase is a thyroid differentiation marker down-regulated in cancer. Endocr. Relat. Cancer **17**(3), 785–796 (2010)
28. O. Young, T. Crotty, R. O'Connell, J. O'Sullivan, A.J. Curran, Levels of oxidative damage and lipid peroxidation in thyroid neoplasia. Head Neck **32**(6), 750–756 (2010)
29. E. Carmeli, A. Bachar, S. Barchad, M. Morad, J. Merrick, Antioxidant status in the serum of persons with intellectual disability and hypothyroidism: a pilot study. Res. Dev. Disabil. **29**(5), 431–438 (2008)
30. A. Saad-Hussein, H. Hamdy, H.M. Aziz, H. Mahdy-Abdallah, Thyroid functions in paints production workers and the mechanism of oxidative-antioxidants status. Toxicol. Ind. Health **27**(3), 257–263 (2011)
31. C. Marcocci, L. Bartalena, Role of oxidative stress and selenium in Graves' hyperthyroidism and orbitopathy. J. Endocrinol. Invest. **36**(10 Suppl), 15–20 (2013)
32. A. Kaur, S. Pandey, S. Kumar, A.A. Mehdi, A. Mishra, Oxidative stress profile in graves' ophthalmopathy in Indian patients. Orbit **29**(2), 97–101 (2010)

33. B. Pereira, L.F. Rosa, D.A. Safi, E.L. Bechara, R. Curi, Control of superoxide dismutase, catalase and glutathione peroxidase activities in rat lymphoid organs by thyroid hormones. J. Endocrinol. **140**(1), 73–77 (1994)
34. A. Swaroop, T. Ramasarma, Heat exposure and hypothyroid conditions decrease hydrogen peroxide generation in liver mitochondria. Biochem. J. **226**(2), 403–408 (1985)
35. V.S. Reddy, S. Gouroju, M.M. Suchitra, V. Suresh, A. Sachan, P.V. Srinivasa Rao, A.R. Bitla, Antioxidant defense in overt and subclinical hypothyroidism. Horm. Metab. Res. **45**(10), 754–758 (2013)
36. M. Kaçmaz, M. Atmaca, A. Arslan, H. Demir, M.F. Ozbay, Oxidative stress in patients with thyroidectomy and thyroparathyroidectomy under replacement therapy. Endocrine. (2014). doi:10.1007/s12020-014-0270-6
37. M. Oncel, A. Kıyıcı, S. Onen, Evaluation of the Relationship Between Ischemia-Modified Albumin Levels and Thyroid Hormone Levels. J. Clin. Lab. Anal. (2014). doi:10.1002/jcla.21789
38. N.I. Krinsky, Mechanism of action of biological antioxidants. Proc. Soc. Exp. Biol. Med. **200**(2), 248–254 (1992)
39. B. Halliwell, J.M. Gutteridge, Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. **186**, 1–85 (1990)
40. U. Resch, G. Helsel, F. Tatzber, H. Sinzinger, Antioxidant status in thyroid dysfunction. Clin. Chem. Lab. Med. **40**(11), 1132–1134 (2002)
41. V. Sundaram, A.N. Hanna, L. Koneru, H.A. Newman, J.M. Falko, Both hypothyroidism and hyperthyroidism enhance low density lipoprotein oxidation. J. Clin. Endocrinol. Metab. **82**(10), 3421–3424 (1997)
42. T. Diekman, P.N. Demacker, J.J. Kastelein, A.F. Stalenhoef, W.M. Wiersinga, Increased oxidizability of low-density lipoproteins in hypothyroidism. J. Clin. Endocrinol. Metab. **83**(5), 1752–1755 (1998)
43. B.T. Kurien, H. Scofield, Autoimmunity and oxidatively modified autoantigens. Autoimmun. Rev. **7**(7), 567–573 (2008)
44. D. Shah, N. Mahajan, S. Sah, S.K. Nath, B. Paudyal, Oxidative stress and its biomarkers in systemic lupus erythematosus. J. Biomed. Sci. **21**, 23 (2014)
45. N.C. Laddha, M. Dwivedi, M.S. Mansuri, A.R. Gani, M. Ansarullah, A.V. Ramachandran, S. Dalai, R. Begum, Vitiligo: interplay between oxidative stress and immune system. Exp. Dermatol. **22**(4), 245–250 (2013)
46. B. Zhang, C. Lo, L. Shen, R. Sood, C. Jones, K. Cusmano-Ozog, S. Park-Snyder, W. Wong, M. Jeng, T. Cowan, E.G. Engleman, J.L. Zehnder, The role of vanin-1 and oxidative stress-related pathways in distinguishing acute and chronic pediatric ITP. Blood **117**(17), 4569–4579 (2011)
47. M.M. Delmastro, J.D. Piganelli, Oxidative stess and redox modulation potential in type 1 diabetes. Clin. Dev. Immunol. **2011**, 593863 (2011)
48. R. Rostami, M.R. Aghasi, A. Mohammadi, J. Nourooz-Zadeh, Enhanced oxidative stress in Hashimoto's thyroiditis: inter-relationships to biomarkers of thyroid function. Clin. Biochem. **46**(4–5), 308–312 (2013)
49. G. Vitale, S. Salvioli, C. Franceschi, Oxidative stress and the ageing endocrine system. Nat. Rev. Endocrinol. **9**(4), 228–240 (2013)
50. P. Mitrou, S.A. Raptis, G. Dimitriadis, Thyroid disease in older people. Maturitas **70**(1), 5–9 (2011)
51. C. Duthoit, V. Estienne, A. Giraud, J.M. Durand-Gorde, A.K. Rasmussen, U. Feldt-Rasmussen, P. Carayon, J. Ruf, Hydrogen peroxide-induced production of a 40 kDa immunoreactive thyroglobulin fragment in human thyroid cells: the onset of thyroid autoimmunity? Biochem. J. **360**(Pt 3), 557–562 (2001)
52. C.L. Burek, N.R. Rose, Autoimmune thyroiditis and ROS. Autoimmun. Rev. **7**(7), 530–537 (2008)
53. B. Poljsak, D. Suput, I. Milisav, Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxid. Med. Cell. Longev. (2013). doi:10.1155/2013/956792
54. B. Halliwell, The wanderings of a free radical. Free Radic. Biol. Med. **46**(5), 531–542 (2009)
55. K. Schlesier, M. Harwat, V. Böhm, R. Bitsch, Assessment of antioxidant activity by using different in vitro methods. Free Radic. Res. **36**(2), 177–187 (2002)
56. R.L. Prior, G. Cao, In vivo total antioxidant capacity: comparison of different analytical methods. Free Radic. Biol. Med. **27**(11–12), 1173–1181 (1999)
57. D. Wang, J.F. Feng, P. Zeng, Y.H. Yang, J. Luo, Y.W. Yang, Total oxidant/antioxidant status in sera of patients with thyroid cancers. Endocr. Relat. Cancer **18**(6), 773–782 (2011)