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Research Article

Chitotriosidase might be a diagnostic marker in patients with hashimoto thyroiditis

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Abstract

Objectives: Hashimoto's thyroiditis (HT) is the most common organ-specific autoimmune disease. The aim of our study was to investigate a possible role of chitotriosidase (Chito) activity as a potential marker of inflammation in diagnosis of patients with HT. In addition, we aimed to determine the levels of inflammation markers such as YKL-40, high sensitivity C-reactive protein (hsCRP) and oxidative stress (OS) parameters such as thiobarbituric acid reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD), GSH-Px to evaluate an association between those inflammatory and OS markers in patients with HT.

Methods: 42 patients with HT and 28 control cases were studied. All cases were euthyroid. Chito activity was measured fluorometrically based on Hollak et al's. method. hsCRP and YKL-40 levels were measured using ELISA. TBARS, SOD, and CAT activities were determined in hemolyzates. GSH-Px activity was determined by a colorimetric assay.

Results: Higher Chito concentrations were observed in patients with HT compared to the control group (p=0.002). YKL-40 levels were detected higher in HT but it was not statistically significant (p=0.810). Chito levels were positively correlated with age and negatively correlated with SOD (r=0.360, p=0.021; r=-0.368, p=0.018). YKL-40 levels were positively correlated with FT3 and SOD in HT (r=0.324, p=0.037; r=0.312, p=0.044) and negatively correlated with age (r=−0.463, p=0.002). SOD levels were negatively correlated with age and positively correlated with YKL-40 (r=−0.371, p=0.016, r=0.312, p=0.044).

Conclusion: We revealed that Chito levels were higher among euthyroid HT patients. This result may point out that Chito levels could be used as a potential marker of inflammation in HT.

Keywords: Catalase, chitotriosidase, glutathione peroxidase, hashimoto thyroiditis, thiobarbituric acid reactive substances, YKL-40

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Hashimoto's thyroiditis (HT) is the most common organ-specific autoimmune disease and is the most common cause of hypothyroidism in iodine-sufficient areas of the world. It affects approximately 10% of the population, especially females, and its prevalence increases with age. It is characterized by gradual thyroid insufficiency, with goitrous or atrophic gland, due to lymphocytic infiltration and autoimmune-mediated damage of the thyroid gland including apoptosis of thyroid epithelial cells [1].

Chitotriosidase (Chito), a chitinase, is a part of the 18-glycosylase family released from activated macrophages and neutrophils into the extracellular fluid as a non-specific inflammatory response [2]. It was demonstrated that Chito activity was increased up to 55 fold in extracts of atherosclerotic tis-

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sue, displaying an association between Chito expression and lipid-laden macrophages inside atherosclerotic vessel wall [3]. It was suggested that Chito activity might indicate the extension of atherosclerosis. Higher Chito activities were detected in patients with ischemic heart disease [4].

YKL-40 is a member of the glycosyl hydrolase protein family and it is also known as the chitinase 3-like protein [5]. It is assumed that it plays a role in cell growth, tissue regeneration and chronic inflammation [6]. It is released by extensive diversity of cells such as neutrophils, endothelial cells, smooth muscle cells, fibroblast-like cells, and cancer cells [7]. The synthesis and secretion of YKL-40 are arranged by growth factors, cytokines, extracellular matrix alterations, stress and drugs [8]. High-sensitivity C-reactive protein (hs-CRP) is a member of inflammatory markers, arranged by pro-inflammatory cytokines. The levels of CRP increase with inflammation [9].

The oxidative stress (OS) can be described as an imbalance in antioxidants and prooxidants, which lead to deterioration of redox signaling and macromolecular damage [10, 11]. It was demonstrated that free radical-mediated reactions played a pivotal role in the pathogenesis of autoimmune diseases [12]. The relationship between increased autoimmune thyroid disease and OS was revealed, however the link between them is still obscure [13, 14]. Increased plasma malondialdehyde (MDA) concentration, the activities of catalase (CAT) and superoxide dismutase (SOD) were detected in patients with HT compared to controls [13]. On the other hand, reactive oxygen species (ROS) and free radicals took part in physiological and pathological processes in the thyroid gland, due to its normal physiological activity [15]. Thyroid cells were shown to have antioxidant protection mechanisms to restrict the action of ROS. Glutathione peroxidase (GSH-Px) could behave as an antioxidant enzyme and preserved the thyroid cells from oxidative injury [16]. It was reported that the existence of autoimmune antibodies played an important role for increased ROS production and enhanced levels of OS markers, whereas thyroid hormone levels had secondary significance [17].

The aim of our study was to investigate a possible role of Chito activity as a potential marker of inflammation in diagnosis of patients with HT. In addition, we aimed to determine the levels of inflammation markers such as YKL-40, high sensitivity C-reactive protein (hsCRP) and OS parameters such as thiobarbituric acid reactive substances (TBARS), CAT, SOD, GSH-Px to evaluate an association between those inflammatory and OS markers in patients with HT. The literature was limited about Chito levels in patients with HT. As far as we know, this is the first study investigating YKL-40 levels in HT, besides Chito levels and its relationship with inflammation markers and OS parameters in patients with HT.

Materials and Methods

Study groups

This case–control study consisted of patients with HT applied to Ege University Faculty of Medicine Endocrinology Department between 2021 and 2022. We collected 42 patients with HT and 28 controls referred to our outpatient clinic. All cases were euthyroid. Exclusion criteria for both groups included, diabetes mellitus, kidney, hepatic or cardiac failure, history of cancer, autoimmune (except HT), inflammatory, or infectious disease. HT was defined with respect to the accepted laboratory and ultrasonographic criteria [18]. 23 patients with HT were taking L-thyroxine treatment; however, the rest of the patients with HT did not receive L-thyroxine treatment and TSH levels of the patients with HT were within the reference ranges. The study protocol was approved by the Ethics Committee of Ege University Faculty of Medicine (22-6.1T/17). Informed consent was obtained from all subjects.

Anthropometric and laboratory measurements

We collected the demographic, clinical and laboratory data from patients' files. Physical examination and anthropometric measurement of all subjects were also obtained. BMI was calculated with this formula: weight (kg)/square meter of height (m^2).

Liver function tests (alanine aminotransferase [ALT]), aspartate aminotransferase [AST]), total cholesterol, triglycerides (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), fasting plasma glucose, fasting insulin, glycated hemoglobin A1c (HbA1c), thyroid stimulating hormone (TSH), free thyroxine (FT4), free tri-iodothyronin (FT3), anti-thyroperoxidase antibody (TPOAb), and anti-thyroglobulin antibody (TgAb) were noted from the patients' files. All routine laboratory tests have been performed with Roche Cobas-8000 autoanalyzer.

Serum samples were separated by centrifugation and stored at −80°C until assay. hsCRP measurements were made using an ELISA method according to the manufacturer's instructions. Serum GSH-Px activity (Elabscience, GSH-Px Activity Assay Kit, Cat# E-BC-K096-S) was determined using a colorimetric assay and YKL-40 (SunredBio, Human YKL-40/CHI3L1 ELISA kit, Cat# 201-12-2064) was determined using a commercially available ELISA kit.

Blood samples with heparin were obtained after overnight fasting (between 8:30 and 9:30 Am). After separation of plasma, the packed erythrocytes were washed twice with 9 g/l NaCl solution and hemolyzed with ice-cold water (1/5, v/v). SOD, CAT activities, and TBARS levels were determined immediately in erythrocyte hemolyzates. The hemoglobin values were measured by Drabkin's method.

SOD activities were measured through a previously described method which was based on the inhibition of autoxidation epinephrine by SOD at 480 nm [19]. The assay was calibrated using purified SOD and one unit of enzyme was defined as the amount of enzyme, which inhibits 50% of autoxidation of epinephrine.

CAT activities were determined as described previously [19] in which the degradation of hydrogen peroxide is recorded spectrophotometrically at 240 nm. One unit of CAT was defined as the amount of enzyme, which decomposed 1 micromol H_2O_2/min under specific conditions. Calculation of enzyme activity was made according to Aebi et al. [20].

Table 1. Demographic, metabolic, and laboratory data of the patient and control groups

Variables	Patients with hashimoto thyroiditis (n=42)	Controls (n=28)	p
Age (years)	39 (23)	48 (16)	**0.016**
Sex, n			
Female	36	13	**<0.001**
Male	6	15	
BMI (kg/m^2)	23.33 (6.03)	25.03 (5.38)	0.097
Smoking, n (%)	8 (19.5)	8 (28.6)	0.381
AST (U/L)	18.00 (6.00)	18.00 (8.00)	0.986
ALT (U/L)	14.00 (7.00)	15.50 (12.00)	0.097
TC (mg/dL)	196.00 (58.00)	196.50 (27.00)	0.787
TG (mg/dL)	88.00 (50.00)	83.00 (70.00)	0.375
HDL-C (mg/dL)	62.50 (18.00)	49.50 (28.00)	**0.019**
LDL-C (mg/dL)	113.00 (53.00)	122.50 (25.00)	0.598
FPG (mg/dL)	85.50 (14.00)	92.00 (8.00)	**0.009**
Fasting insulin (mU/L)	9.45 (6.86)	11.20 (11.16)	0.072
HbA1c (%)	5.20 (0.53)	5.30 (0.40)	0.382
HOMA-IR	1.99 (1.46)	2.55 (2.51)	**0.043**
TSH (mU/L)	1.76 (1.87)	1.77 (1.09)	0.439
FT4 (ng/dL)	1.23 (0.37)	1.25 (0.33)	0.649
FT3 (ng/L)	3.01 (0.65)	3.21 (0.58)	**0.031**
TPOAb (IU/mL)	89.25 (177.00)	15.00 (0.00)	**<0.001**
TgAb (IU/mL)	35.45 (193.75)	15.00 (0.00)	**<0.001**
CHITO (nmol/mL/h)	40.65 (41.74)	21.88 (21.56)	**0.002**
YKL-40 (ng/mL)	3.02 (2.75)	1.70 (2.16)	0.810
hs-CRP (mg/dL)	0.11 (0.36)	0.12 (0.22)	0.853
TBARS(nmol/gHB)	9.53 (3.13)	9.92 (3.11)	0.114
Catalase (U/gHB)	4715.85 (2873.12)	5632.25 (4010.05)	0.233
SOD (U/g HB)	554.90 (125.80)	581.65 (124.92)	0.097
GSH-Px (U/g HB)	235.25 (75.05)	263.80 (99.10)	0.067

P<0.05 was significant. The results were presented as median (interquartile range). The p values were obtained through statistical analyses using Mann-Whitney U test or chi-square test. BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; FPG: Fasting plasma glucose; HbA1c: Hemoglobin A1c; HOMA-IR: Homeostasis model assessment-insulin resistance; TSH: Thyroid-stimulating hormone; FT4: Free thyroxine; FT3: Free tri-iodothyronin; TPOAb: Anti-thyroperoxidase antibody; TgAb: Anti-thyroglobulin antibody; CHITO: Chitotriosidase; hs-CRP: High sensitive-C reactive protein; TBARS: Thiobarbituric Acid Reactive Substances; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase. The standard reference range as follows: TSH 0.27–4.2 mU/L, FT4 0.89–1.76 ng/dL, FT3 2–4.4 ng/L, TPOab 0–34 IU/mL, TgAb 0–115 IU/mL, FPG 60–110 mg/dL, HOMA-IR<2.5 mmol/L, HbA1c 4.8–5.9%.

TBARS levels were measured by the method reported previously [21]. The hemolysates were incubated with TBA-working solution (0.12 M TBA in 15% TCA and 1% HCl) for 30 min at 95°C. TBARS concentrations was calculated using a calibration curve constructed from 1,1,3,3 tetra ethoxy propan. Chito activity was measured fluorometrically based on Hollak et al's. method [22]. Briefly, 10 µL serum was incubated with 4-methylumbelliferyl-β-d -NN, N′-triacetylchitotriose (Sigma Chemical, St Louis, MO, USA) as substrate in Na/acetate buffer (0.25 mol/L, pH 5.5), at 37°C for 1 h. The reaction was stopped with 0.1 mol/L ethylenediamine. Fluorescent (excitation 365 nm and emission 450 nm) of 4-methylumbelliferone was measured with a fluorometer (Thermo Fisher scientific/Specrofluorometer/ Varıoskan, MA, USA). 4-Methylumbelliferone was used for calibration curve. The enzyme activity was evaluated as nmol/mL/h. The CV with in-run and CVbetween-run are 8.8% and 16%, respectively.

Statistical analysis

We conducted a priori and a *post hoc* power analysis with the program G*Power 3.1.9.4 [23]. A priori power analysis indicated that a total sample size of 56 (28 per group) would be sufficient to detect a large effect (d=0.7) with a power of 0.80 and an alpha of 0.05. The *post hoc* analyses revealed the statistical power for this study for detecting a large effect of 0.83 with a power of 0.95 for the sample size 70.

Descriptive statistics for continuous variables were calculated with median and inter-quartile range; frequencies and percentages were given for categorical variables. The Shapiro–

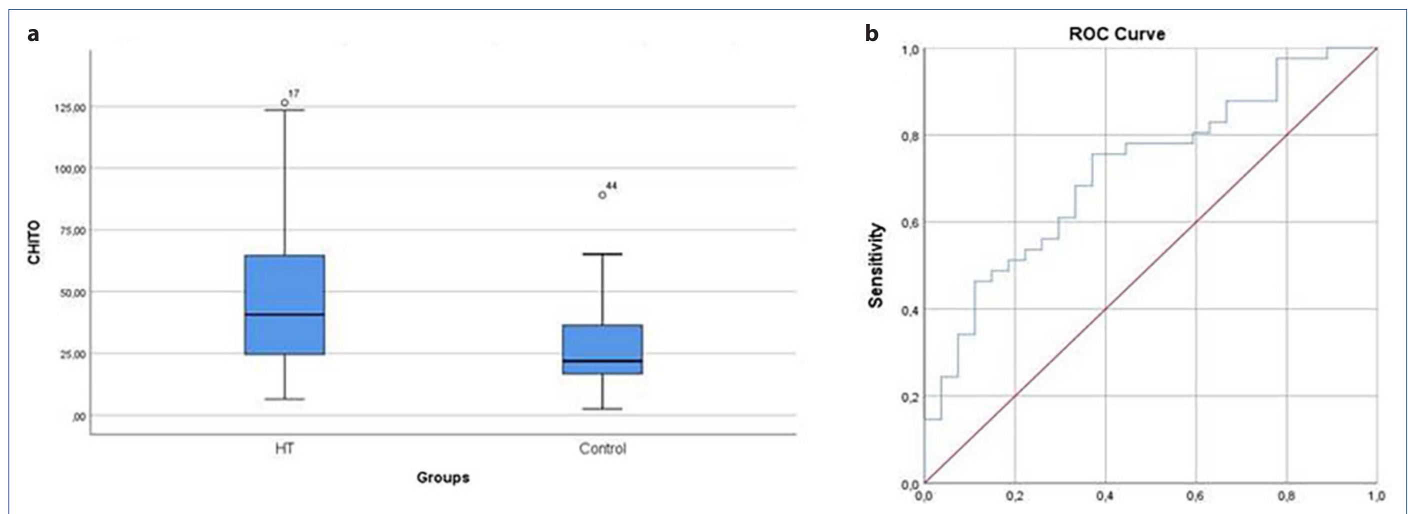

Figure 1. Significant difference in Chito levels in patients with HT. (a) The median Chito level in the patients with HT was significantly higher than in the control group (p=0.002). (b) The receiver operating characteristic (ROC) curve demonstrated the area under the curve of 0.719 (95% CI: 0.597–0.841, p<0.002).

HT: Hashimoto's thyroiditis.

Wilk test was used to check the normality assumption of the continuous variables. The Wilcoxon rank-sum (Mann–Whitney U) test was performed to compare continuous variables between the HT and controls. The Pearson Chi-square test was used for the analysis of categorical variables in two groups. The relationship between variables in patients with HT was examined through Spearman's rank correlation. A receiver operating characteristic curve (ROC) was performed to identify the optimal cut-off value for HT patients-controls differentiation in Chito. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS version 25.0 (Chicago, IL, USA).

Results

Clinical and laboratory characteristics of the study population

The demographics and clinical characteristics of all patients and control subjects were demonstrated in Table 1. All cases were euthyroid (median TSH level 1.76 mU/L in HT vs. 1.77 mU/L in controls, p=0.439). The median age of HT and control group was 39 and 48 years, respectively. A history of smoking existed in 19.5% of the patients with HT and 28.6% of the control group (p=0.381). BMI was not significantly different between two groups (p=0.097). The levels of TPOAb and TgAb were significantly different between patients with HT and controls (p<0.001 and p<0.001, respectively).

FPG levels were significantly different among groups (p=0.009). HOMA-IR was observed to be decreased in patients with HT compared to the control group (p=0.043). HbA1c levels were found lower in HT compared to the control group, but the difference was not statistically significant (p=0.382). We found that AST, ALT, and LDL-C were not statistically different between groups (p=0.986, p=0.097, and p=0.598, respectively). HDL-C levels were statistically higher in patients with HT (p=0.019). In addition, FT3 levels were lower in patients with HT (p=0.031).

Levels of inflammation markers, OS markers

The results for inflammation markers are displayed in Table 1. Although higher Chito and YKL-40 concentrations were observed in patients with HT compared to the control group, increase in Chito concentrations were statistically significant (p=0.002, p=0.810, Fig. 1a). On the other hand, hsCRP, TBARS levels and CAT, SOD and GSH-Px activities were not statistically significant different in patients with HT compared to control group (p=0.853, p=0.114, p=0.233, p=0.097, and p=0.067, respectively).

Correlation of inflammation markers, OS markers

Correlation analyses of Chito, YKL-40, hsCRP, TBARS, CAT, SOD, and GSH-Px with metabolic, anthropometric, and laboratory parameters are displayed presented in Table 2. Chito levels were positively correlated with age and negatively correlated with SOD (r=0.360, p=0.021; r=–0.368, p=0.018). YKL-40 levels were positively correlated with FT3 and SOD in patients with HT (r=0.324, p=0.037; r=0.312, p=0.044) and negatively correlated with age (r=–0.463, p=0.002). Furthermore, hsCRP levels were significantly related with BMI, FPG, fasting insulin, LDL-C, HDL-C, HOMA-IR in the patients with HT (r=0.564, p<0.001; r=0.394, p=0.016; r=0.327, p=0.048; r=0.344, p=0.037; r=-0.496, p=0.002; r=0.427, p=0.008). Moreover, TBARS levels were statistically and significantly correlated with BMI, TC, and FT4 (r=0.519, p<0.001; r=0.325, p=0.036; r=–0.346,p=0.025). Besides, CAT levels were only associated with TgAb (r=–0.384, p=0.030). SOD levels were negatively correlated with age and positively correlated with YKL-40 (r=–0.371, p=0.016, r=0.312,

Table 2. Correlation analysis of inflammation and oxidative parameters with metabolic, anthropometric, and laboratory parameters in patients with hashimoto thyroiditis

	Chito		YKL-40		hsCRP		TBARS		Catalase		SOD		GSH-Px	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Age (years)	0.360	0.021*	−0.463	0.002*	0.260	0.120	0.280	0.073	−0.057	0.757	−0.371	0.016*	−0.162	0.304
BMI (kg/m²)	0.054	0.739	−0.256	0.102	0.564	<0.001*	0.519	<0.001*	−0.206	0.259	−0.136	0.392	−0.198	0.209
Insulin (U/L)	−0.304	0.053	0.091	0.567	0.394	0.016*	−0.003	0.986	−0.233	0.199	0.169	0.286	−0.076	0.634
FPG (mg/dL)	0.155	0.332	−0.260	0.096	0.327	0.048*	0.250	0.111	−0.061	0.740	−0.211	0.180	0.090	0.569
HbA1c	0.179	0.263	−0.203	0.198	0.258	0.124	0.216	0.170	−0.084	0.648	−0.150	0.341	0.002	0.989
TC	0.182	0.254	−0.262	0.094	0.300	0.071	0.325	0.036*	−0.176	0.336	−0.203	0.198	−0.090	0.570
LDL	0.187	0.242	−0.204	0.194	0.344	0.037*	0.379	0.013	−0.142	0.439	−0.159	0.314	−0.103	0.517
HDL	0.134	0.403	0.133	0.402	−0.496	0.002*	−0.206	0.191	0.149	0.415	0.082	0.606	0.327	0.034*
TG	0.042	0.793	−0.172	0.275	0.281	0.092	0.198	0.208	−0.164	0.369	0.000	1.000	−0.253	0.107
HOMA-IR	−0.208	0.192	0.010	0.950	0.427	0.008*	0.046	0.773	−0.194	0.289	0.096	0.544	−0.039	0.808
TSH	0.081	0.613	0.063	0.691	0.212	0.208	0.164	0.299	0.184	0.312	0.119	0.454	−0.197	0.210
FT4	−0.176	0.271	−0.186	0.237	−0.161	0.341	−0.346	0.025*	−0.001	0.998	−0.120	0.448	0.161	0.309
FT3	−0.261	0.100	0.324	0.037*	−0.076	0.657	−0.143	0.367	0.294	0.103	0.195	0.217	0.190	0.229
TPOAb	0.010	0.949	−0.035	0.828	−0.033	0.845	−0.208	0.187	−0.042	0.818	−0.054	0.732	0.077	0.627
TgAb	−0.043	0.790	−0.146	0.356	−0.211	0.209	0.031	0.848	−0.384	0.030*	−0.144	0.362	−0.132	0.405
Chito	–	–	−0.203	0.203	−0.139	0.413	0.208	0.193	0.240	0.193	−0.368	0.018	0.011	0.947
YKL-40	−0.203	0.203	–	–	−0.259	0.122	0.109	0.494	0.231	0.204	0.312	0.044*	0.098	0.539
hsCRP	−0.139	0.413	−0.259	0.122	–	–	−0.061	0.719	0.056	−0.153	−0.153	0.366	−0.053	0.755
MDA	0.208	0.193	0.109	0.494	−0.061	0.719	–	–	−0.084	0.648	0.020	0.899	−0.236	0.133
Catalase	0.240	0.193	0.231	0.204	0.056	0.782	−0.084	0.648	–	–	−0.199	0.274	0.305	0.090
SOD	−0.368	0.018*	0.312	0.044*	−0.153	0.366	0.020	0.899	−0.199	0.274	–	–	0.030	0.851
GSH-Px	0.011	0.947	0.098	0.539	−0.053	0.755	−0.236	0.133	0.305	0.090	0.030	0.851	–	–

*: A p value of<0.05 was considered significant. Chito: Chitotriosidase; hsCRP: High sensitive C reactive protein; TBARS: Thiobarbituric acid reactive substances; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; r: Spearman's correlation coefficient; BMI: Body mass index; FPG: Fasting plasma glucose; HbA1c: Hemoglobin A1c; TC: Total cholesterol; LDL: Low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol, TG: Triglyceride; HOMA-IR: Homeostasis model assessment of insulin resistance; TSH: Thyroid-stimulating hormone; FT4: Free thyroxine; FT3: Free tri-iodothyronin; TPOAb: Anti-thyroperoxidase antibody; TgAb: Anti-thyroglobulin antibody; MDA: Malondialdehyde.

p=0.044). GSH-Px levels were positively correlated with HDL in patients with HT (r=0.327, p=0.034).

ROC analysis

The optimal cutoff point for Chito was 24.50 ng/mL. The sensitivity and specificity of serum Chito for the diagnosis of HT were 0.756% and 0.630%, respectively. ROC analysis presented the area under the curve of 0.719 (95% CI: 0.597–0.841, p<0.002) (Fig. 1b). Based on this value, we assumed that Chito could be a good option and might have a potential to differentiate HT.

Discussion

In the current study, we detected inflammation markers; OS markers in patients with HT. Inflammation markers such as Chito, YKL-40 and hsCRP, OS markers such as TBARS, CAT, SOD, and GSH-Px were evaluated. While Chito activities were higher in HT than controls, there was no difference in other parameters in HT compared to controls. In the present study, we used a wide panel of markers of OS in HT, because only one biomarker did not demonstrate OS [24]. Hypothyroidism was detected to be related with increased OS and caused to decrease free anti-oxidant defense systems [16, 25]. Limited studies were detected in the literature associated with oxidative status in euthyroid HT patients [26, 27]. Morawska et al. [17] demonstrated that oxidative damage to salivary proteins and lipids were detected in salivary glands of women with HT in euthyreosis.

We reported that Chito levels were higher in HT than in the controls. This may be resulted from the stimulation of macrophage activation in HT [28]. The optimal cutoff point for Chito may indicate the possibility of euthyroid HT patients to enter hypothyroidism in the near future. In contrast with our study, Erdal et al. [29] demonstrated no change in Chito activity in patients compared to controls. Since their study revealed that treatment with L-thyroxine in subclinical hypothyroidism did not decrease the serum hsCRP levels but increased the serum Chito levels, they suggested that L-thyroxine might induce macrophage activation. It was demonstrated that the extent of inflammatory condition was related with Chito activity in inflammatory diseases such as type 1 diabetes mellitus [30], and Chito was assessed as a part of innate immunity [31], it was expected to find an increase in Chito activity in autoimmune thyroid disease. Recently, it was demonstrated that inhibition of Chito levels led to a decrease in the number of granulomas and the expression of sarcoidosis-related genes [32].

Increased YKL-40 levels have been demonstrated in a broad spectrum of disorders and pathological situations such as inflammatory diseases [33]. Many studies revealed that increased levels of YKL-40 in inflammatory disease such as pyoderma gangrenosum and inflammatory bowel diseases [33, 34]. Tizaoui et al. [35] evaluated 21 articles on the YKL-40 levels in rheumatoid arthritis (RA), and they assumed that YKL-40 could be a diagnostic marker of RA and YKL-40 might show the disease activity. Since HT had a similar nature with autoimmune diseases, we expected to observe high levels of YKL-40 in HT patients. Although there was an increase (3.5 vs. 1.7) in YKL-40 levels compared to control, it was non-significant. It might be explained with the negative correlation between age and YKL-40 levels in our study group where age of control group was lower than HT patients.

ROS causes lipid peroxidation and ROS-mediated oxidation of cell membrane lipids results in the formation of lipid peroxidation products, for example, MDA [36]. MDA is used as an indicator of OS. In addition, it was reported that increased MDA levels, proof of enhanced lipid peroxidation, were detected in hypothyroidism. After reaching euthyroid state there was a decrease in MDA levels in the patients with hypothyroidism [37]. Furthermore, it was revealed that endogenous MDA levels were not different in HT with subclinical hypothyroidism and healthy controls. However, MDA levels were increased in HT with overt hypothyroidism [38]. In the present study, TBARS levels were not different in HT compared to controls. This might have been resulted from euthyroid situation of our patients.

CAT is necessary to degrade hydrogen peroxide at higher concentrations. Decreased CAT activities led to hydrogen peroxide-induced apoptosis of thyroid cells in patients with HT [39]. Baskol et al. [37] demonstrated that SOD activity was not found different in patients with hypothyroidism before treatment when compared to control group. CAT and SOD activities were lower in patients with HT, but they were not statistically significant in our study.

GSH-Px is responsible for enzymatic reduction of catalyzes hydrogen peroxide [40]. GSH-Px provides the protection of thyrocytes from oxidative damage and regulation of thyroid hormone biosynthesis [41]. Nourbakhsh et al. [42] revealed that GSH-Px activity was detected similar in patients with HT and normal subjects. They speculated that it might be resulted from normal selenium status. As mentioned above, Lassoued et al. [13] revealed decreased GSH-Px activity in HT. In contrast, some reports demonstrated enhanced GSH-Px activity in hypothyroidism [14, 43]. In our study, GSH-Px activities were lower in patients with HT but they were not statistically significant.

Our study had some limitations. It had a cross-sectional study design. Moreover, it is impossible to assess the causal relationships between inflammation markers, OS markers and the evolution of thyroid function tests. In addition, the lack of association between inflammation markers and OS parameters might be due to the fact that some patients in HT group received L-thyroxine treatment.

In conclusion, our data showed that Chito levels were higher in euthyroid HT patients. This result may point out that Chito levels could be used as a potential marker of inflammation in HT. Further prospective studies are needed on this issue.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by The Ege University Faculty of Medicine Ethics Committee (No: 22-6.1T/17, Date: 23/06/2022).

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References

1. Jin B, Wang S, Fan Z. Pathogenesis markers of Hashimoto's disease-a mini review. Front Biosci (Landmark Ed) 2022;27(10):297. [CrossRef]
2. Mack I, Hector A, Ballbach M, Kohlhäufl J, Fuchs KJ, Weber A, et al. The role of chitin, chitinases, and chitinase-like proteins in pediatric lung diseases. Mol Cell Pediatr 2015;2(1):3. [CrossRef]
3. Boot RG, van Achterberg TA, van Aken BE, Renkema GH, Jacobs MJ, Aerts JM, et al. Strong induction of members of the chitinase family of proteins in atherosclerosis: chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. Arterioscler Thromb Vasc Biol 1999;19(3):687–94. [CrossRef]
4. Cetin Sanlialp S, Nar G, Senol H. Clinical significance of chitotriosidase in outpatients with advanced heart failure. J Investig Med 2021;69(3):736–41. [CrossRef]
5. Hector A, Kormann MS, Mack I, Latzin P, Casaulta C, Kieninger E, et al. The chitinase-like protein YKL-40 modulates cystic fibrosis lung disease. PLoS One 2011;6(9):e24399. [CrossRef]
6. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, et al. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol 2011;73:479–501. [CrossRef]
7. Zhao T, Su Z, Li Y, Zhang X, You Q. Chitinase-3 like-protein-1 function and its role in diseases. Signal Transduct Target Ther 2020;5(1):201. [CrossRef]
8. Kzhyshkowska J, Mamidi S, Gratchev A, Kremmer E, Schmuttermaier C, Krusell L, et al. Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway. Blood 2006;107(8):3221–8. [CrossRef]
9. Stanciu AE, Serdarevic N, Hurduc AE, Stanciu MM. IL-4, IL-10 and high sensitivity-CRP as potential serum biomarkers of persistent/recurrent disease in papillary thyroid carcinoma with/without Hashimoto's thyroiditis. Scand J Clin Lab Invest 2015;75(7):539–48. [CrossRef]
10. Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol 2008;295(4):C849–68. [CrossRef]
11. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol 2015;4:180–3. [CrossRef]
12. Wang G, Wang J, Fan X, Ansari GA, Khan MF. Protein adducts of malondialdehyde and 4-hydroxynonenal contribute to trichloroethene-mediated autoimmunity via activating Th17 cells: dose- and time-response studies in female MRL+/+ mice. Toxicology 2012;292(2-3):113–22. [CrossRef]
13. Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, et al. A comparative study of the oxidative profile in Graves' disease, Hashimoto's thyroiditis, and papillary thyroid cancer. Biol Trace Elem Res 2010;138(1-3):107–15. [CrossRef]
14. Rostami R, Aghasi MR, Mohammadi A, Nourooz-Zadeh J. Enhanced oxidative stress in Hashimoto's thyroiditis: inter-relationships to biomarkers of thyroid function. Clin Biochem 2013;46:308–12. [CrossRef]
15. Schmutzler C, Mentrup B, Schomburg L, Hoang-Vu C, Herzog V, Köhrle J. Selenoproteins of the thyroid gland: expression, localization and possible function of glutathione peroxidase 3. Biol Chem 2007;388(10):1053–9. [CrossRef]
16. Reddy VS, Gouroju S, Suchitra MM, Suresh V, Sachan A, Srinivasa Rao PV, et al. Antioxidant defense in overt and subclinical hypothyroidism. Horm Metab Res 2013;45(10):754–8. [CrossRef]
17. Morawska K, Maciejczyk M, Popławski Ł, Popławska-Kita A, Kretowski A, Zalewska A. Enhanced salivary and general oxidative stress in hashimoto's thyroiditis women in euthyreosis. J Clin Med 2020;9. [CrossRef]
18. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 2014;13(4-5):391–7. [CrossRef]
19. Sözmen EY, Tanyalçin T, Onat T, Kutay F, Erlaçin S. Ethanol induced oxidative stress and membrane injury in rat erythrocytes. Eur J Clin Chem Clin Biochem 1994;32(10):741–4. [CrossRef]
20. Aebi H, Wyss SR, Scherz B, Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. Eur J Biochem 1974;48(1):137–45. [CrossRef]
21. Sözmen EY, Sözmen B, Girgin FK, Delen Y, Azarsiz E, Erdener D, et al. Antioxidant enzymes and paraoxonase show a co-activity in preserving low-density lipoprotein from oxidation. Clin Exp Med 2001;1(4):195–9. [CrossRef]
22. Hollak CE, van Weely S, van Oers MH, Aerts JM. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J Clin Invest 1994;93(3):1288–92. [CrossRef]
23. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-91.
24. Lushchak VI. Classification of oxidative stress based on its intensity. EXCLI J 2014;13:922–37.
25. Resch U, Helsel G, Tatzber F, Sinzinger H. Antioxidant status in thyroid dysfunction. Clin Chem Lab Med 2002;40(11):1132–4.
26. Baser H, Can U, Baser S, Yerlikaya FH, Aslan U, Hidayetoglu BT. Assesment of oxidative status and its association with thyroid autoantibodies in patients with euthyroid autoimmune thyroiditis. Endocrine 2015;48(3):916–23. [CrossRef]
27. Ates I, Yilmaz FM, Altay M, Yilmaz N, Berker D, Güler S. The relationship between oxidative stress and autoimmunity in Hashimoto's thyroiditis. Eur J Endocrinol 2015;173(6):791–9.

28. Cao Y, Sun Y, Jin X, Li X, Chen L, Qiu Y. TIM4/NLRP3 axis participates in the effects of M1 macrophages on inflammatory factor release, apoptosis and cell adhesion in thyroid follicular cells. Exp Ther Med 2022;24(5):679. [CrossRef]
29. Erdal M, Sahin M, Saglam K, Hasimi A, Uckaya G, Yarpuz MY, et al. Increased serum chitotriosidase activity following restoration of euthyroidism in patients with subclinical hypothyroidism. Intern Med 2008;47:1309–14. [CrossRef]
30. Cutaș A, Drugan C, Roman G, Rusu A, Cătană CS, Achimaș-Cadariu A, et al. Evaluation of Chitotriosidase and Neopterin as Biomarkers of Microvascular Complications in Patients with Type 1 Diabetes Mellitus. Diagnostics (Basel) 2021;11(2):263.
31. van Eijk M, van Roomen CP, Renkema GH, Bussink AP, Andrews L, Blommaart EF, et al. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int Immunol 2005;17(11):1505–12. [CrossRef]
32. Dymek B, Sklepkiewicz P, Mlacki M, Güner NC, Nejman-Gryz P, Drzewicka K, et al. Pharmacological Inhibition of Chitotriosidase (CHIT1) as a Novel Therapeutic Approach for Sarcoidosis. J Inflamm Res 2022;15:5621–34. [CrossRef]
33. Jankowska-Konsur A, Łyko M, Rubas K, Nowicka-Suszko D, Maj J, Szepietowski JC. Chitinase-3-like Protein 1 (YKL-40): A New Biomarker of Inflammation in Pyoderma Gangrenosum. Acta Derm Venereol 2022;102:adv00646. [CrossRef]
34. Koutroubakis IE, Petinaki E, Dimoulios P, Vardas E, Roussomoustakaki M, Maniatis AN, et al. Increased serum levels of YKL-40 in patients with inflammatory bowel disease. Int J Colorectal Dis 2003;18(3):254–9. [CrossRef]
35. Tizaoui K, Yang JW, Lee KH, Kim JH, Kim M, Yoon S, et al. The role of YKL-40 in the pathogenesis of autoimmune diseases: a comprehensive review. Int J Biol Sci 2022;18(9):3731–46. [CrossRef]
36. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 1991;91(3C):14S–22S. [CrossRef]
37. Baskol G, Atmaca H, Tanriverdi F, Baskol M, Kocer D, Bayram F. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment. Exp Clin Endocrinol Diabetes 2007;115(8):522–6. [CrossRef]
38. Öztürk Ü, Vural P, Özderya A, Karadağ B, Doğru-Abbasoğlu S, Uysal M. Oxidative stress parameters in serum and low density lipoproteins of Hashimoto's thyroiditis patients with subclinical and overt hypothyroidism. Int Immunopharmacol 2012;14(4):349–52. [CrossRef]
39. Björkman U, Ekholm R. Hydrogen peroxide degradation and glutathione peroxidase activity in cultures of thyroid cells. Mol Cell Endocrinol 1995;111(1):99–107. [CrossRef]
40. Brigelius-Flohé R, Flohé L. Is there a role of glutathione peroxidases in signaling and differentiation? Biofactors 2003;17(1-4):93–102. [CrossRef]
41. Chiu-Ugalde J, Wirth EK, Klein MO, Sapin R, Fradejas-Villar N, Renko K, et al. Thyroid function is maintained despite increased oxidative stress in mice lacking selenoprotein biosynthesis in thyroid epithelial cells. Antioxid Redox Signal 2012;17(6):902–13. [CrossRef]
42. Nourbakhsh M, Ahmadpour F, Chahardoli B, Malekpour-Dehkordi Z, Hosseini-Fard SR, Doustimotlagh A, et al. Selenium and its relationship with selenoprotein P and glutathione peroxidase in children and adolescents with Hashimoto's thyroiditis and hypothyroidism. J Trace Elem Med Biol 2016;34:10–4. [CrossRef]
43. Nanda N, Bobby Z, Hamide A. Oxidative stress and protein glycation in primary hypothyroidism. Male/female difference. Clin Exp Med 2008;8(2):101–8. [CrossRef]