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ISCHEMIA MODIFIED ALBUMIN AND THIOL/DISULFIDE BALANCE IN PATIENTS WITH HASHIMOTO'S THYROIDITIS

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Hashimoto thyroiditis is a common cause of goiter and acquired hypothyroidism in individuals residing in areas of no iodine deficiency. The fact that the structure of serum albumin exhibits changes in ischemic conditions has paved the way for the discovery of a new serum cardiac ischemia marker, Ischemia Modified Albumin. The other one, thiol/disulphide homeostasis, plays an important part in antioxidative protection, detoxification, cell growth, and apoptosis. In this study, we aimed to investigate both the relationship between Thiol/Disulphide homeostasis and Ischemia Modified Albumin in patients diagnosed with Hashimoto's Thyroiditis. A total of 70 Hashimoto's thyroiditis patients and 50 healthy ones were included in this study. Age, gender, thyroid-stimulating hormone (TSH), anti-thyroid peroxidase (TPO), anti-thyroglobulin (TG) levels were recorded. Ischemia Modified Albumin and thiol-disulphid homeostasis parameters were measured through automated spectrophotometric methods. The ages of individuals included in the study ranged from 35 to 58 years. The native thiol/total thiol were found to be significantly lower in Hashimoto patients when compared to those enrolled in the control group ($P < 0.05$), whereas the Ischemia Modified Albumin, disulphide, native thiol, total thiol, disulphide/native thiol, and disulphide/total thiol were found to be significantly higher in Hashimoto patients when compared to those in the control ($P < 0.05$). Increased Ischemia Modified Albumin, native and total thiol, and disulphide levels are related to increased oxidative stress. Although Ischemia Modified Albumin and Thiol-disulphide defense are important oxidative indicators in Hashimoto's Thyroiditis, many determinants are known to be involved in this process.

Ke y w o r d s: Hashimoto's thyroiditis, ischemia modified albumin, thiol-disulphide homeostasis.

Hashimoto's Thyroiditis (HT), which is an organ-specific autoimmune disease and known also as chronic lymphocytic thyroiditis, is a frequently encountered thyroid disease. It was first defined by Dr. Hakaru Hashimoto in the consequence of histopathology examination of the thyroid tissue of four cases in 1912 [1]. Annually, its incidence in males is 0.08% and 0.35% in females. It is seen more frequently in females than males and it is most frequently diagnosed between the ages of 30 and 50 [2].

The course of autoimmune Hashimoto's Thyroiditis is a process that continues in the form of thyroid destruction rather than thyroid stimulation. The breakdown of immune regularity ends up with influenced immune cells in the thyroid gland. The lymphocytes become overly sensitive against the thyroid antigens and the formed antibodies react

with the thyroid antigens such as trio globulin and thyroid peroxidase. General destruction takes place in the lymphocyte infiltration and the normal thyroid tissue architecture [3,4]. One of the reasons that trigger this destruction in the thyroid tissue is oxidative stress. As a result of increased oxidative stress, inflammation takes place in the thyroid tissue. And this chronic inflammation of the thyroid is called Hashimoto's disease [5]. The ischemia-modified albumin (IMA) and the thiol/disulphide are among the systemic indications of oxidative stress [6]. The last amino acid in the albumin structure can bind up to the passing heavy metals. The reasons such as the damage to the free radicals and disintegration of the cell membrane reduce the bonding of these metals to the N-terminals of albumin and this newly formed damaged albumin is called the IMA [7,8]. The IMA is a deeply studied indicator in the last several years, giving prospects for the future.

The thiol/disulphide defense has an important place in cell growth, apoptosis, and detoxification [9]. In the conducted studies, the etiopathogenic role of this balance was investigated in many different diseases such as Hashimoto's thyroiditis, polycystic ovary syndrome, prediabetes, Crimean-Congo hemorrhagi fever. It is believed that this balance has a significant role in immune etiopathogenesis and the Thiol/Disulphide imbalance triggers the disease through oxidative stress and tissue inflammation [10].

In this study, we aimed to evaluate ischemia-modified albumin and thiol/disulphide balance in Hashimoto's Thyroiditis and healthy subjects. As seen in our literature review, this is the first study to evaluate disulphide, disulphide/native thiol, disulphide/total thiol with IMA, and the correlation among them in patients with Hashimoto's Thyroiditis.

Material and Methods

Study population. This study was carried out with the individuals admitted to the Internal Diseases Clinic and approved by the local ethics committee by the principles of the 2008 Helsinki Declaration. Patient numbers were determined according to power analysis. Ethical Approval: This research is approved by the Research Ethics committee (28.08.2019,2019-33). A total of 70 Hashimoto's Thyroiditis patients and 50 healthy volunteers between 35-58 years of age were included in the study. Hashimoto's thyroiditis was diagnosed when the anti-TG and/or anti-TPO were positive in the serum together with the presence of parenchymal heterogeneity on thyroid ultrasonography (USG).

Assay for thiol/disulphide balance. Thiol-disulphide homeostasis level was measured by the automated spectrophotometric method described by Erel and Neselıoglu (2014) [11, 12]. Biochrom Libra UV/VIS and Roche automatic analyzer (Roche) were used during these measurements. The data were calculated by dividing the difference between the total thiol and the native thiol groups by two. After calculating each parameter (natural thiol, total thiol, and disulphide amount); disulphide/total thiol ratios, native thiol/total thiol ratios, and disulphide/native thiol ratios were determined.

Assay for ischemia modified albumin levels. Serum IMA level was determined by modifying the method developed by Bar-Or et al. (2000) [13]. A new test called the Albumin cobalt binding test (ACB) was utilized which is cobalt addition of the structural change occurring due to ischemia on the album and the spectrophotometric measurement of unbound cobalt. The principle of the method is briefly as follows: The concentration of IMA is determined by adding a known amount of cobalt to the serum sample and spectrophotometrically measuring the unbound cobalt ions at 470 nm using dithiothreitol (DTT). Since IMA concentrations were not standard, the absorbance was given in absorbance units (ABSU). For each sample at 470 nm, the absorbance of both the sample blank and the samples were measured and the difference was assigned as the IMA value of that sample.

Statistical analysis. The findings of the study were statistically evaluated using IBM SPSS Statistic 22.0 (IBM Co., Armonk, NY, USA). All data were given as mean ± standard deviation. The statistical significance level was defined as 0.05. The normal distribution of data was evaluated with Shapiro-Wilk tests. Numerical variables were shown as mean ± SD. The One-Way ANOVA test was preferred for parametric tests due to the normal distribution of data. The Mann-Whitney U test was used for non-parametric data. The correlation between parameters was observed via the Pearson correlation test.

Results

While the native thiol [SH], total thiol [SH-SS], disulphide [-SS], and disulphide/native thiol (SS/SH) were found to be significantly higher in Hashimoto patients compared to those in the controls ($P < 0.05$),

Table. The work parameters of healthy individuals and patients with Hashimoto's Thyroiditis

Parameters	Patients			Control			U	*P*
	Mean±SD	SEM	Min.-Max.	Mean±SD	SEM	Min.-Max.		
Native thiol (µmol/l)	476.6 ± 3.4	8.8	407.0-531.0	429.1 ± 69.5	13.9	312.0-551.0	509.000	0.020*
Total thiol (µmol/l)	567.5 ± 76.5	15.6	486.0-709.0	454.4 ± 76.9	15.4	321.0-585.0	90.000	0.000*
Disulphide (µmol/l)	29.8 ± 8.2	1.6	20.5-52.0	20.5 ± 5.2	1.0	11.5-30.0	101.000	0.000*
Disulphide/ Native thiol	0.063 ± 0.01	0.0	0.04-0.10	0.049 ± 0.0	0.0	0.02-0.08	174.000	0.012*
Disulphide/Total thiol	0.053 ± 0.01	0.0	0.03-0.10	0.046 ± 0.1	0.0	0.03-0.08	219.000	0.105
Native thiol/Total thiol	0.85 ± 0.12	0.0	0.57-0.99	0.98± 0.21	0.1	0.57-1.53	207.00	0.063
IMA (ABSU)	0.76 ± 0.80	0.16	0.65-0.95	0.64 ± 0.12	0.02	0.27-0.83	143.500	0.002*

IMA, Ischemia modified albumin; ABSU, absorbance units U, Mann Whitney U test; *$P < 0.05$

whereas native thiol (SH) /total thiol (SH-SS) were found to be lower in the patients ($P > 0.05$) (Table 1). Also, disulphide (-SS)/ total thiol (SH-SS) were found to be higher in patients ($P > 0.05$).

The ischemia-modified albumin levels were found to be significantly higher in Hashimoto patients compared to those in the control group ($P < 0.05$, $P = 0.002$) (Table 1).

A positive correlation was found between the IMA – native thiol and IMA data – total thiol data at the $P < 0.05$ level and between the IMA - disulphide data at the $P < 0.01$ level ($r = 0.363$, $r = 0.232$ and $r = 301$, respectively). However, a weak negative correlation was found between native thiol and total thiol ($r = -0.230$).

Discussion

In this study, we aimed to demonstrate thiol/ disulphide homeostasis and IMA in Hashimoto's thyroiditis patients. As seen in our literature review, this is the first study to present increased disulphide, disulphide/native thiol, disulphide/total thiol, IMA, and the correlation among them in patients with Hashimoto's Thyroiditis.

In Hashimoto's thyroiditis, slowed down energy metabolism leads to reduced consumption of O_2, low basal metabolic rate, the elevation of lipidemia, and resultant metabolic suppression [14, 15].

As recently demonstrated, structural changes occur in serum albumin in ischemic conditions, paving for the discovery of a new serum marker [16]. As we mentioned in the general description, we carried out this study to investigate any changes occurring in IMA, a marker of ischemia, in thyroid dysfunction and to examine potential effects of the thyroid gland, an endocrine gland that affects the consumption of O2 and the metabolism, on IMA. Oxygen used by the organism is associated with the changes in metabolic mechanisms that are primarily responsible for heart ischemia in thyroid functions. IMA is evaluated in cardiac ischemia and is regarded as an important marker. Studies demonstrate that IMA concentration is high in pulmonary embolism, mesenteric ischemia, peripheral artery occlusion, deep vein thrombosis, acute ischemic events, stroke, and acute cardiac arrest [17]. Therefore, it is considered that IMA can be used as a diagnostic marker. Studies are showing that IMA levels may be elevated in untreated hypothyroidism patients as a marker of oxidative stress [18-20]. We detected that IMA values of HT patients were significantly higher

compared to controls. Similarly, in some studies, it was found that there was a rise in IMA levels in subclinical hypothyroidism or hypothyroidism [20-22]. In addition, some studies revealed a high IMA under hypoxia and oxidative stress [23]. Our results suggest that there is a strong relationship between increased IMA and hypoxia levels and oxidative stress in the HT process.

The human body has potent natural defense systems to use against the destructive effects of free oxygen radicals that prevent reactions caused by free radicals, which result in cellular injury. This mechanism aims at ensuring cellular protection and it is manifested by the synthesis of protective enzymes and chemical compounds in a sufficient concentration under normal circumstances [24]. Overproduction of oxidative products takes part in the etiology of many diseases, including but not only limited to cancer, chronic renal failure [CRF], diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, ischemia-reperfusion injury, and immune diseases. Oxidative stress is defined as the loss of the balance between oxidative and anti-oxidative mechanisms [23]. Thiol compounds are organic substances that have reductive features, which assume the important task of defending the body against oxidative stress [25]. Oxidative products that are synthesized in the organism, such as reactive oxygen species, are reduced by transferring excess electrons to the compounds that contain thiol, while thiol groups are simultaneously oxidized [26]. Oxidation of thiol groups causes the formation of disulphide bonds. However, it is a reversible reaction, and the disulphide bonds can be reduced to thiol groups, thus ensuring homeostasis. And this homeostasis plays an important role in the anti-oxidative process. Recent studies have reported that impairment of homeostasis leads to CRF, DM, cardiovascular diseases, cancer, chronic inflammatory joint diseases, and various neurodegenerative disorders [25]. Information on various normal and abnormal biochemical processes could be obtained by measuring the thiol-disulphide homeostasis [27]. Several studies have reported that thiol/disulphide balance deteriorates in diseases characterized by intense inflammation and oxidative stress [28-30]. We measured thiol/disulphide homeostasis in this study. We detected that native thiol, total thiol, disulphide values, and disulphide/native thiol, disulphide/total thiol ratios of HT patients were significantly higher, while native thiol/total thiol ratio of these patients were significantly lower compared to controls. Similarly, in the study of Koca et al., (2019), the disulphide, disulphide/native thiol, disulphide/total thiol levels were found higher in a patient with hypoparathyroidism [31]. Mengen et al., (2020) reported high levels of thiol/disulphide homeostasis and IMA levels in obesity [32]. The disulphide, disulphide/native thiol, disulphide/total thiol levels were found higher in a patient with acute and chronic idiopathic thrombocytopenic purpura in the study of Kar et al., (2021) [33].

Conclusions. We concluded that native thiol, total thiol, and disulphide values were higher in HT patients compared to healthy controls. In our study, we concluded that thiol-disulphide defense could be a biomarker in HT patients and that IMA data of these biomarkers have a correlation. Although IMA is an important indicator in these patients, many determinants are known to be involved in this process.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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БАЛАНС РІВНІВ МОДИФІКОВАНОГО ІШЕМІЄЮ АЛЬБУМІНУ ТА ТІОЛУ/ ДИСУЛЬФІДУ У ХВОРИХ НА ТИРЕОЇДИТ ХАШИМОТО

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Тиреоїдит Хашимото є частою причиною зобу та набутого гіпотиреозу у людей, які проживають в районах, де немає йододефіциту.Той факт, що структура сироваткового альбуміну змінюється за умов ішемії, проклав шлях до відкриття нового сироваткового маркера ішемії серця – модифікованого ішемією альбуміна

(MIA). Інший показник, тіол/дисульфідний гомеостаз, відіграє важливу роль в антиоксидантному захисті, детоксикації та апоптозі. У цьому дослідженні ми мали на меті дослідити взаємозв'язок між тіол/дисульфідним гомеостазом та рівнем IMA у сироватці крові пацієнтів з діагнозом тиреоїдит Хашимото. У дослідження було включено 70 пацієнтів із тиреоїдитом Хашимото та 50 здорових пацієнтів віком від 35 до 58 років, було зареєстровано їх стать та рівень тиреотропного гормону (ТТГ), антитиреоїдної пероксидази (ТПО), антитиреоглобуліну (ТГ). Рівень MIA та параметри тіол-дисульфідного гомеостазу вимірювали автоматизованими спектрофотометричними методами. Виявлено, що співвідношення нативний тіол/загальний тіол було значно нижчим у хворих пацієнтів порівняно з включеними до контрольної групи ($P < 0,05$), тоді як рівні IMA, альбуміну, дисульфіду, нативного та загального тіолу, співвідношення дисульфід/нативний тіол були значно вищим у пацієнтів з хворобою Хашимото порівняно з контролем ($P < 0,05$). Зроблено висновок, що тіол-дисульфідний гомеостаз корелює з рівнем IMA в сироватці крові і може бути біомаркером у пацієнтів з ГТ.

Ключові слова: тиреоїдит Хашимото; модифікований ішемією альбумін; тіол-дисульфідний гомеостаз.

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