Total Antioxidant Capacity, Salivary Catalase, and Superoxide Dismutase in Hashimoto's Thyroiditis Patients

Shahin Nosratzehi[1], Mahin Nosratzehi[1], Ebrahim Alijani[2], Abolfazl Payandeh[3], Tahereh Nosratzehi[4], Abdulmannan Charvaei[5]

[1] Department of Internal Medicine, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

[2] Department of Immunology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

[3] Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran

[4] Department of Oral and Maxillofacial Medicine, School of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran

[5] School of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran

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Abstract- Hashimoto's thyroiditis (HT) is one of the common causes of hypothyroidism. Although various factors are involved in its development, recently the role of oxidative stress in its pathogenesis has been known. The present study aimed to investigate the level of total antioxidant capacity (TAC), catalase (CAT), and salivary superoxide dismutase (SOD) in patients with HT compared with the control group. The present case-control design included patients aged 18-80 years suffering from HT referred to the endocrine clinic. Eligible patients were selected by the available sampling method. Complete unstimulated saliva was collected under a rest state in a comfortable room between 10:00 AM and 12:00 AM and a checklist was used to collect data. The chi-square, t-test, and Mann-Whitney U tests were used for data analysis using SPSS 22 software. The mean age of the participants was 36.55±9.37 years (range: 20-56). The two groups were the same in terms of age and gender (P>0.05). The findings indicated that the difference in the means CAT between the two groups was 22.63 which was strongly and statistically significant (P<0.001). In this study, the level of TAC and SOD in Hashimoto's thyroid patients was decreased and the level of CAT was increased. These initial findings show that oxidative stress can be associated with Hashimoto's thyroid disease or the possibility of developing this disease increase.

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Keywords: Hashimoto's thyroiditis; Antioxidant; Superoxide dismutase; Oxidative stress; Saliva

Introduction

Hashimoto's thyroiditis (HT) is a multifactorial disease and one of the most prevalent thyroid autoimmune disorders (1). HT, also called "chronic lymphocytic thyroiditis " or "autoimmune thyroiditis" was first described by Hakura Hashimoto in 1912 (2). In HT patients, the thyroid gland is hardened and enlarged with a characteristic pathological appearance (3). The presence of thyroid peroxidase autoantibodies (anti-TPO) is an important biomarker for the diagnosis of HT (4). The prevalence of HT in the general population is 10%, and women are 5-10 times more susceptible than men (5). The occurrence of HT is related to age, ethnicity, environmental factors, and gender. Excessive dietary iodine intake has been significantly suggested as an environmental trigger of HT. High consumption of iodine has a series of inhibitory effects on secretion and the biosynthesis of thyroid hormone. It has also shown that excessive consumption of iodine reduces cellular antioxidant activity in experimental animal models (6). Furthermore, recent prospective researches have revealed that even a minimal increase in iodine levels in iodine-replete areas is correlated with an increased

Corresponding Author: T. Nosratzehi
Department of Oral and Maxillofacial Medicine, School of Dentistry, Zahedan University of Medical Sciences, Zahedan, Iran
Tel: +98 9152345423, E-mail address: taherehnosratzahi@yahoo.com

incidence of HT (7). The pathogenesis of HT is marked by the progressive destruction of thyroid cells and mechanisms including cell apoptosis and oxidative stress (8,9).

The participation of the oxidative stress mechanism in the pathogenesis of HT is still unclear (10). The characteristic of HT is the presence of blocking autoantibodies (TSH-R), which reduces the secretion of triiodothyronine (T3) and thyroxine (T4) (11). In HT, B and T lymphocytes, which are stimulated against thyroid peroxidase (TPO) and thyroglobulin, lead to inflammation and destruction of the thyroid. B and T lymphocytes may result in the overproduction of reactive oxygen species (ROS) through the enzyme NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (12).

In thyroiditis, free radicals and ROS constantly generate and participate in pathological and physiological conditions (13). For example, hydrogen peroxide (H2O2) is essential for making thyroid hormones (14). However, some laboratory experimental studies showed that H2O2 also affects the process of cell death (15,16). Cells develop an extensive set of antioxidant activities to neutralize the effect of ROS. The antioxidants catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) have been identified in the thyroid gland (13). SOD catalyzes the conversion of two superoxide molecules into H2O2 and oxygen (17). CAT and GPX mainly remove H2O2 as the primary factor in the main antioxidant process (18).

In physiological conditions, ROS molecules that are crucial for thyroid hormone biosynthesis (during iodine oxidation and binding to amino acids) in epithelial cells of thyroid gland. However, H2O2 levels may increase excessively in the presence of certain stimuli including inflammation (activation of B and T lymphocytes), radiation, medications, chemicals, and excessive iodine intake (19-21). In this excessively increased H2O2 environment, thyrocytes undergo apoptosis, necrosis, and destruction as a result (22). Another condition that can lead to an increase in ROS in HT is the decrease in the synthesis of antioxidant enzymes due to the decline in thyroid hormone levels (10,13). In addition, it is also known that hyperlipidemia, which is caused by a low level of thyroid hormone, also leads to a rise in ROS (23).

Many oxidizing factors that have the ability to generate excessive production of ROS can lead to a change in the redox balance of the oral cavity (24). Previous studies showed that saliva and plasma antioxidants play an important role in the form of the antioxidant barrier both in the mouth and in the whole body (25). Salivary peroxidase and CAT together neutralize the H2O2 formed in the mutagenesis reactions by SOD catalytic activity (26). The failure of the antioxidant activities may lead to oral cavity diseases including precancerous lesions, periodontitis and cancer. Evidence have revealed the change in salivary antioxidant barrier and the effect of oxidative stress result in the salivary gland dysfunction in the course of autoimmune diseases: systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus, Sjogren's syndrome, psoriasis vulgaris and multiple sclerosis (26-30). In general, a change in the total antioxidant capacity (TAC) in the saliva and an increase in the oxidative damage of salivary glands detected in the saliva of patients with autoimmune disorders (31).

Few studies assessed salivary redox homeostasis in HT, it seems necessary to evaluate the antioxidant capacity of saliva and the role of oxidative stress in causing salivary gland problems during HT. It should be mentioned that abnormality in the composition and amount of saliva secreted has a negative effect on the health of the mouth and the whole body (31). Therefore, understanding the mechanisms that lead to salivary gland dysfunction, evaluating the antioxidant salivary activities, and the role of oxidative stress in causing salivary gland dysfunction during HT seems essential. The present study aimed to investigate the level of TAC, CAT, and salivary SOD in patients with HT compared with the control group.

Materials and Methods

The present case-control design included patients aged 18-80 years suffering from HT referred to the endocrine clinic of a university-affiliated hospital in Zahedan, southeastern Iran. The sample size was calculated based on the formula of statistical test for assessing means equality between two parallel groups (32). According to the study by Lasoued *et al*., (33), considering the significance level of 0.05, power of 0.95, means difference (ε) of 0.41, and nonresponse probability of 0.10, 30 patients with HT and 30 healthy individuals as a control group were computed and included in the study. Eligible patients were selected by the available sampling method.

The HT was defined as follows; decreased total Thyroxine (T4) <4.5 μg/dL (normal range, 4.5-12 μg/dL), in association with elevated thyroid stimulating hormone (TSH) ≥10 mIU/L, and positive anti-TPO Ab>16 IU/ml (normal range up to 16 IU/ml) or

antithyroglobulin >100 mIU/L (normal range up to 100 mIU/L). If the initial tests were positive for hypothyroidism, to rule out transient hypothyroidism, the tests were repeated three months later, and if the hypothyroidism was approved, the subjects were included in the study.

Controls referred to the Department of Oral Diseases School of Dentistry were frequently matched with the case groups in terms of age and gender. Inclusion criteria were; 1. Not having gingivitis, periodontitis, or an active focus of odontogenic infections for subsequent examinations. 2. Not taking any meditablecation that can affect the secretion of saliva (antidepressants or antihypertensive drugs) or its redox processes (antioxidant supplements, vitamins). Subjects with any autoimmune diseases (rheumatoid arthritis, type 1 diabetes, psoriasis, scleroderma, Sjogren's syndrome, lupus, etc.), subjects with depression, and subjects with smoking, alcohol consumption, stimulants, and incomplete checklists were excluded.

A written informed consent was signed by the participants. Complete unstimulated saliva was collected under a rest state in a comfortable room between 10:00 AM and 12:00 AM. Subjects must be at least 120 minutes before taking samples to avoid eating, drinking, smoking, and using mouthwash. 5 ml of non-irritating saliva sample was collected by pouring saliva out of the mouth in the absence of chewing movements. One of the best methods for collecting whole saliva is the spitting method. After confirmation, the saliva samples were centrifuged (2500 g, 10 minutes), and the supernatant was immediately separated from the saliva and stored at -70° C for further evaluation (34). Laboratory measurements of saliva concentration, total antioxidant, CAT, and salivary SOD levels were determined by Enzyme-linked immunosorbent assay (ELISA) using Navand biological kits (Nactaz™ CAT Activity Assay Kit, NazdoxTM SOD Activity Assay Kit) with 95% sensitivity.

The data were analyzed using SPSS version 22. Descriptive statistics (e.g., frequency, percentage and mean ± standard deviation (SD) were calculated. Given the normal distribution of the studied variables including the TAC and the salivary SOD, the parametric t-test was employed. The chi-square test was used to compare the frequency of sex in patients with HT and healthy controls. Also, due to the non-normal distribution of CAT activity, a non-parametric Mann-Whitney U test was used. The significance level was set at 0.05.

Results

The mean age of the participants was 36.55±9.37 years (range: 20-56). Table 1 shows that the two groups were the same in terms of age and gender (P>0.05). The Comparison of the TAC, CAT, and salivary SOD in patients with HT and healthy control group were summarized in Table 2. The findings indicated that the difference in the means CAT between the two groups was 22.63 which was strongly and statistically significant (P<0.001).

Table 1. The comparison of the mean age and gender of subjects in HT and healthy control groups

Variables		Patients with HT N=30	Healthy controls N=30	*P*
Age (yr)		35.04±9.62 [a]	37.89±9.11	0.283
Gender	**Female**	21 (70)	19 (63)	0.514
	Male	9 (30)	11 (37)	

[a] Data were summarized as mean±SD or frequency (percent)
Abbreviations: HT, Hashimoto's thyroiditis

Table 2. The Comparison of the TAC, CAT, and SOD in patients with HT with the control group

Variables mean±SD	Patients with N=30	Healthy controls N=30	CI (95%) Lower	CI (95%) Upper	*P*
TAC	257.67±90.06	268.35±55.25	-53.70	32.34	0.62
CAT	33.06±11.43	10.43±4.6	18.32	26.94	<0.001*
SOD	47.50±26.78	60.04±24.29	-26.90	1.83	0.09

*Significant
Abbreviations: HT, Hashimoto's thyroiditis; TAC, total antioxidant capacity; CAT, catalase; SOD, salivary superoxide dismutase

Discussion

The present study aimed to investigate the level of TAC, CAT, and salivary SOD in patients with HT compared with controls. Our results showed that the mean salivary CAT in the patients was significantly

higher than in the healthy controls. The present findings also revealed that the mean of TAC and SOD in patients with HT was lower than in the healthy control group, but these differences were not significant.

The present study in agreement with previous studies concerning salivary redox homeostasis indicated that CAT activity was higher in patients with HT than in the healthy controls (35,36). Similarly, numerous studies have indicated that the TAC and SOD activity decreased in the patients with HT (36). For instance, the results of studies by Ates *et al*., (37) showed that oxidative stress parameters increase continuously during all stages of HT. Also, Morawska and her colleagues (35) conducted a study to assess salivary redox homeostasis in patients with HT and showed a decline in antioxidant activity (82%) and enhanced generation of ROS as well as excessive oxidative agents in the plasma of patients with HT in euthyreosis. Moreover, medical literature about the TAC in hypothyroidism and hyperthyroidism agree with our findings (38-40).

The present study is one of the few studies that investigated CAT activity levels in HT. The result of a study conducted by Lassoued and his colleagues showed that CAT activities increased significantly only for papillary thyroid cancer (PTC) and HT patients, and the results of this study are in line with the present study (33). In a study done by Naazeri *et al*., (41) on an Iranian sample with hypo-and hyperthyroidism, the enhanced activity of CAT and SOD are also reported, and the results of the study are in agreement with our findings. The results of several studies showed that CAT activity increased in patients with hyperthyroidism (42,43). However, Pasupathi and Sahoo colleagues showed that the CAT activity in patients with hypothyroidism was lower than in the controls, which is inconsistent with the present study (44,45). The precise cause for the decrease in CAT activity, an enzyme known for its antioxidant properties, in this particular ailment, remains elusive. More studies are needed in this field.

The results of the present study showed that the decreased level of salivary SOD in patients with HT in compared with controls. In the study of Pasupathi *et al*., (44), SOD activity was lower in patients with hypothyroidism than in controls, which is in line with our study. The reduced levels of these antioxidants are due to increased oxidative stress in patients. In agreement with our findings, Resch *et al*., showed decreased SOD activity in patients with hypothyroidism in comparison with the controls.

The present study had limitations, which can be pointed to the small sample size in the studied groups and also that only two parameters of several parameters of oxidative stress were measured.

In this study, the level of total antioxidant and superoxide dismutase in patients with HT was decreased and the level of catalase was increased. These initial findings show that oxidative stress can be associated with HT disease or the possibility of developing this disease increase.

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