Salivary Glutathione Peroxidase Level in Patients with Hashimoto's Thyroiditis

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Article type	ABSTRACT
Research Paper	**Introduction:** The role of oxidative stress in pathogenesis and autoimmune diseases is well known. According to the function of the enzyme glutathione peroxidase via its antioxidant role in the body, the aim of this study was to investigate the level of salivary glutathione peroxidase in patients with Hashimoto's thyroiditis compared to a control group. **Materials & Methods:** In this case-control study, 30 patients with Hashimoto's thyroiditis and 30 healthy subjects were studied as a control group. The two studied groups were the same in terms of age and gender. Five ml of total unstimulated saliva of the subjects was collected under resting conditions in a quiet room between 10 a.m. and 12 noon (spitting method). The amount of glutathione peroxidase in the saliva samples of these subjects was measured using the ELISA method. The results were analyzed using Mann-Whitney and chi-square statistical tests at a significance level of 0.05. **Results:** The results showed that the mean age of the participants was 36.96 ± 8.85 years. Totally, 71.7% of the participants were women with a mean age of 37.74 ± 8.29 years and 28.3% were men with a mean age of 35 ± 9.24 years. The level of salivary glutathione peroxidase was significantly lower in patients with Hashimoto's thyroiditis (13.26 ± 13.55) than in the control group (24.95 ± 18.06, $P<0.002$). **Conclusion:** The level of salivary glutathione peroxidase in patients with Hashimoto's thyroiditis was significantly reduced than normal. Foods rich in antioxidants, especially glutathione, are recommended. **Keywords:** Saliva, Glutathione Peroxidase, Hashimoto Disease, Thyroid

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Introduction

Hashimoto's thyroiditis (HT) can be caused by a variety of factors including inflammation, viral infections, genetic and environmental influences, like iodine supplements, stress, and infections. [1-4] HT is an autoimmune disease characterized by lymphocyte infiltration and destruction of the thyroid gland and leads to hypothyroidism. Molecular similarity, in which the immune system mistakenly targets the thyroid gland due to the similarity between viral antigens and thyroid antigens, is one proposed mechanism for the development of HT. [5]

Other potential causes include immune dysregulation, damage to thyroid cells caused by T cells, and apoptosis. Additionally, oxidative stress has been closely associated with HT. Studies indicate that patients with HT often have an imbalance between oxidants and antioxidants in their bodies resulting in increased stress. [4] In addition, oxidative stress is closely related to HT. Studies have shown that patients with HT. have an imbalance between oxidants and antioxidants, which leads to increased oxidative stress. [6, 7] Furthermore, studies have found that salivary glands in HT patients experience disruptions in homeostasis with decreased secretion and heightened levels of damage to proteins and lipids. [8]

Various salivary biomarkers indicating stress such as catalase and peroxidase activities as well as protein and lipid oxidation products have been shown to be significantly elevated in HT patients' saliva samples.[9] Furthermore, there is a connection, between thyroid autoantibodies and oxidative stress indicators in individuals with HT. It has been observed that higher levels of stress are related to stages and overt hypothyroidism in HT patients. [10] These findings suggest that oxidative stress plays a role, in the pathogenesis and progression of HT, and oxidative stress parameters can potentially be used as biochemical markers to predict disease progression.

The level of salivary glutathione peroxidase (GPx) in HT has not been specifically addressed in the medical literature. However, several studies have evaluated oxidative stress and antioxidant defense in HT patients. One study showed that the concentration of glutathione in the saliva of HT patients was significantly reduced compared to healthy subjects. [6] Another study investigated the association between a GPx-1 genetic variant and the risk of HT but did not directly measure salivary GPx levels. [11] In addition, a study on salivary function in HT patients without dry mouth reported no significant differences in salivary gland function parameters between HT and control groups. [12]

Therefore, while salivary GPx levels were not specifically addressed, these studies suggest that oxidative stress and antioxidant defense mechanisms may be altered in the saliva of HT patients. More research is needed to investigate salivary GPx levels in HT. Therefore, this study was conducted to investigate the level of salivary GPx in patients with HT compared to the control group.

Materials & Methods

The present case-control study included patients aged 18 to 80 years who suffered from HT and were referred to the endocrine clinic of a university hospital in Zahedan in southeastern Iran. This study was approved by the Ethics Committee of Zahedan University of Medical Sciences (ethical number: IR.ZAUMS.REC.1400.237). Eligible patients were selected according to the available sampling method. The cases were 30 patients with HT and 30 individuals as a control group. Sample size calculation was based on the comparison of the mean between two independent groups' formula with a significance level of 5% and power of 80%, including control individuals from the general population.

The control subjects were referred to the Department of Oral Diseases of the Faculty of Dentistry and were often identical to the cases in terms of age groups and gender. Inclusion criteria were: 1. No gingivitis, periodontitis, or an active focus of odontogenic infection for the subsequent examinations. 2. No intake of medication that could influence saliva secretion (antidepressants or antihypertensive medication) or redox processes (antioxidant supplements, vitamins). Subjects with autoimmune diseases (rheumatoid arthritis, type 1 diabetes, psoriasis, scleroderma, Sjogren's syndrome, lupus, etc.), subjects with depression, and subjects who smoke, alcohol consumption, stimulants, and incomplete checklists were excluded.

The participants have signed a written declaration of consent. HT diagnosis was based on the clinical symptoms of hypothyroidism and the anti-TPO values. HT was defined as follows: decreased TT4 <4.5 µg/dL (reference value, 4.5-12 µg/dL), in conjunction with elevated TSH ≥10 mIU/L and positive anti-TPO Ab>16 IU/mL (up to 16 IU/mL) or anti-Tg (up to 100 mIU/L). The saliva samples were taken before the start of treatment in HT patients. Complete, unstimulated saliva was collected at rest in a comfortable room between 10:00 am and 12:00 noon. Subjects were not allowed to eat, drink, smoke, or use mouthwash for at least 120 minutes before sample collection. Five ml of a non-irritating saliva sample was collected by pouring the saliva from the mouth without chewing. One of the best methods for collecting whole saliva is the spitting method. After confirmation, the saliva samples were centrifuged (2500g, 10 minutes), and the supernatant was immediately separated from the saliva and stored at -70°C for further analysis. Laboratory measurements of GPx levels in saliva were determined by an enzyme-linked immunosorbent assay (ELISA) with biological kits from Navand (Nagpix™ GPx Activity Assay Kit) with a sensitivity of 95%.

The data were analyzed using SPSS version 22. Descriptive statistics (e.g., frequency, percentage, and mean ± standard deviation (SD)), the chi-square, and Mann-Whitney U tests were employed. The normality of the data was checked using the Shapiro-Wilk test and quantile-quantile plots. The significance level was set at 0.05.

Results

Of 60 participants, 71.7% were female. The mean age of all subjects was 36.96±8.85 years (Range: 20-55). The mean (±SD) age of men and women were 37.47± 8.29 and 35±9.24 years, respectively. Table 1 shows that the two groups were the same in terms of age groups and gender (P > 0.05). The Comparison of the GPx in patients with HT and the control group was summarized in Table 2. The results of the Mann-Whitney U test indicated that the difference between the mean values of GPx patients and controls was 5.61, which was statistically significant (P = 0.002).

Table 1. The Comparison of age and gender of subjects in HT and control groups.

Characteristics N (%)	Patients with HT* N=30	Controls N=30	P-Value
Age (year)			
20-30	9(30.0)	6(20.0)	
31-40	10(33.3)	16(53.3)	0.293
41and above	11(36.7)	8(26.7)	
Gender			
Female	23 (76.7)	20 (66.7)	
Male	7 (23.3)	10 (33.3)	0.390

Table 2. The Comparison of GPx in patients with HT and the control group.

GPx mean±SD	N	Mean±SD	Median	Min	Max	P-Value
Patients with HT	30	13.26±13.55	9.99	6.01	80.36	
Controls	30	24.95±18.06	15.60	6.18	66.10	0.002*
Total	60	19.10±16.89	11.18	6.01	80.36	

GPx: Glutathione Peroxidase; HT: Hashimoto's thyroiditis; SD: Standard Deviation.

Discussion

According to the results, the level of salivary GPx in patients with HT was significantly lower than in the control group. Our result was consistent with some previous studies [6] comparing salivary glutathione peroxidase levels in HT patients to controls that reveal significant differences in oxidative stress markers. A study by Morawska (2020) indicates that HT patients exhibit lower levels of glutathione and total antioxidant potential, alongside higher oxidative stress indicators in saliva compared to healthy controls. [6] Furthermore, salivary gland function appears compromised in HT patients, with alterations in salivary flow rates and pH levels noted. [11] Moreover, while some studies suggest that salivary function may remain intact in HT patients without xerostomia, the presence of thyroid autoantibodies correlates with salivary dysfunction. [12] Overall, these findings indicate the potential of salivary biomarkers, including glutathione peroxidase, in assessing oxidative stress in autoimmune conditions like HT. [13]

On the other hand, the review of the medical literature on the studies of salivary GPx levels in patients with HT has shown that some studies have reported different results that are not consistent with the results of our study. Gerenova (2007) reported that GPx activity in erythrocytes was significantly higher in hypothyroid HT patients, suggesting a potential compensatory mechanism. [14] These findings are not consistent with the study of Rao (2010), who found no significant difference in the levels of salivary C-reactive protein, a marker of inflammation, between HT patients and the control group. [15] Müssig (2012) also found that the presence of thyroid peroxidase antibodies, a hallmark of HT, was associated with poorer physical and psychological well-being. [16] These studies collectively suggest that the relationship between salivary GPx levels and HT is still controversial and may be influenced by various factors such as thyroid function and the presence of specific antibodies. Research on HT and autoimmune disease faces several key challenges including the lack of knowledge regarding autoantigens, the low frequency of autoreactive cells, and difficulties in accessing affected tissues, which complicate the identification of biomarkers and therapeutic targets. Additionally, the heterogeneity of disease presentations and the presence of comorbid autoimmune conditions can complicate diagnosis, leading to potential misdiagnoses of participants in various studies. Several studies have shown that oxidative stress and antioxidant defense play an important role in the pathophysiology of hypothyroidism. [17, 18]

Overt and subclinical hypothyroidism is associated with a lack of antioxidant defense, which is characterized by a decrease in superoxide dismutase activity, a decrease in the level of plasma iron-reducing ability (FRAP), a decrease in glutathione (GSH), and an increase in GPx activity. [18] This imbalance can lead to increased lipid peroxidation and contribute to disease severity. [18] Similarly, long-term hemodialysis patients also show increased oxidative stress, as indicated by increased malondialdehyde (MDA) and oxidized glutathione (GSH), decreased plasma GPx, and decreased GSH. [19] Research on salivary GPx levels in HT revealed significant demographic patterns, particularly concerning age and gender. Most research indicated a strong female predominance, with studies showing that approximately 88.9% of HT patients are women. Female patients often exhibit higher levels of anti-thyroid antibodies. [20] The age distribution highlights that the peak incidence occurs in the 36–45-year-old age group, while

another study indicates a dramatic increase in HT cases among older patients, particularly those aged 41-50. Furthermore, significant correlation between aging and increased HT incidence. [20] These findings suggest that both age and gender are critical factors in understanding the relationship between salivary GPx levels and Hashimoto's thyroiditis.

Limitations of this study include the small sample size and the lack of further antioxidant testing. Due to the small sample size, no comparisons were made between men and women. Future researchers are recommended to conduct a study with a large sample and a group of patients with hyperthyroidism.

Conclusion

According to the results of the present study, it can be seen that the level of salivary GPx in HT patients was significantly lower than in control individuals. Therefore, it is recommended to increase the consumption of foods rich in antioxidants, especially glutathione, or antioxidant supplements to protect against various injuries.

Funding

Conflicts of Interest

There is no conflict of interest to declare.

Author's Contribution

Shahin Nosratzehi designed the study and supervised the collection of the data. Mostafa Shokri collected the data. Abolfazl Payandeh analyzed the data. Mahin Nosratzehi wrote the original draft and collaborated on the manuscript. Ebrahim Alijani & Tahereh Nosratzehi discussed the results and commented on the manuscript. All authors contributed to the final version of the manuscript.

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