Turk J Endocrinol Metab. 2020;24:321-326

Serum Levels of 8-OHdG in Patients with Hashimoto's Thyroiditis

Hashimoto Tiroiditi Olan Hastalarda Serum 8-OHdG Düzeyleri

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Abstract

Objective: A growing body of evidence shows a close relationship between oxidative stress and autoimmune conditions such as Hashimoto's thyroiditis (HT). Among different markers of oxidative damage, 8-hydroxydeoxyguanosine (8-OHdG) is a ubiquitous marker and broadly used in research studies. Therefore, this study aimed to measure the level of 8-OHdG in the sera of patients with HT and healthy control (HC) participants. **Material and Methods:** In this study, patients were diagnosed with clinical (n=23) and subclinical (n=25) hypothyroidism because of HT and compared with 35 healthy participants. 8-OHdG was measured using enzyme-linked immunosorbent assay in patients and HC participants. **Results:** No significant difference was observed in the mean serum 8-OHdG levels between patients and HC participants. **Conclusion:** The results of this preliminary study do not support the fact that the serum level of 8-OHdG is a biomarker of oxidative stress in patients with HT. However, more detailed studies are needed to reveal the exact role of 8-OHdG in this autoimmune disease of the thyroid gland.

Keywords: 8-hydroxydeoxyguanosine; Hashimoto's thyroiditis; oxidative stress

Özet

Amaç: Giderek artan sayıda kanıt, oksidatif stres ile Hashimoto tiroiditi (HT) gibi otoimmün durumlar arasında yakın bir ilişki olduğunu göstermektedir. Oksidatif hasarın farklı belirteçleri arasında, 8-hidroksideoksiguanozin (8-OHdG) yaygın bir belirteçtir ve araştırma çalışmalarında geniş ölçüde kullanılmaktadır. Bu nedenle, bu çalışma HT hastalarının ve sağlıklı kontrol (SK) grubunun serumlarında 8-OHdG düzeyini ölçmeyi amaçlamıştır. **Gereç ve Yöntemler:** Bu çalışmada hastalara HT nedeniyle klinik (n=23) ve subklinik (n=25) hipotiroidizm tanısı konulmuş ve 35 sağlıklı katılımcı ile karşılaştırılmıştır. 8-OHdG, hastalarda ve SK grubunda enzime bağlı immünosorbent testi kullanılarak ölçülmüştür. **Bulgular:** Hastalar ve SK grubu arasında ortalama serum 8-OHdG düzeylerinde anlamlı bir fark izlenmemiştir. **Sonuç:** Bu ön çalışmanın sonuçları, HT hastalarında serum 8-OHdG düzeyinin oksidatif stresin bir biyobelirteci olduğu gerçeğini desteklememektedir. Bununla birlikte, tiroid bezinin bu otoimmün hastalığında 8-OHdG'nin tam rolünü ortaya çıkarmak için daha detaylı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: 8-hidroksideoksiguanozin; Hashimoto tiroiditi; oksidatif stres

Introduction

Hashimoto's thyroiditis (HT) is characterized by diffuse lymphocytic infiltration of the thyroid gland and elevated level of antithyroid-specific autoantibodies. It is more prevalent among women, and its incidence has increased significantly in recent years (1). Although autoimmunity plays a pivotal role in

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Peer review under responsibility of Turkish Journal of Endocrinology and Metabolism.

Received: 10 Jul 2020 **Received in revised form:** 29 Oct 2020 **Accepted:** 05 Nov 2020 **Available online:** 10 Dec 2020

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DOI: 10.25179/tjem.2020-77613

the pathogenesis of HT, the underlying mechanisms for the initiation and progression of aberrant immune responses are not completely understood (2).

In recent years, several studies suggested a dynamic relationship between the immune system and oxidative stress. Oxidative stress has been shown to play crucial roles in the regulation of specific immunity and inflammatory responses (3,4). Therefore, any disturbance in the oxidant-antioxidant balance can influence immune activity. Under normal conditions the production of reactive oxygen species and free radicals is necessary for thyroid hormonogenesis; (5) however, the overproduction of oxygen radicals damages thyrocytes (6).

Oxidative stress causes damage to four major macromolecules within the cells (proteins, nucleic acids, such as DNA and RNA, carbohydrates, and lipids). The resulting oxidation products from each biomolecule can then be used to assess the direct or indirect index of oxidative stress. Oxidative damage to DNA has been proposed as a novel and crucial factor that may underlie the pathogenesis of various autoimmune or inflammatory diseases. 8-hydroxydeoxyguanosine (8-OHdG) is a major form of the oxidative DNA damage product formed by the attack of oxygen radicals on 2'-deoxyguanosine. It can pair with adenine and cause a G: C→T: A transversion mutation (7). The major mammalian enzyme for removing 8-OHdG from DNA is 8-oxoguanine-DNA glycosylase. Although this enzyme prevents the accumulation of oxidative products of DNA damage, large deletion mutations may also arise during the nucleotide excision repair pathway for removing 8-OHdG from DNA (8).

In recent years, much attention has been focused on investigating the putative role of oxidative stress in the pathogenesis of autoimmune thyroid disorders such as HT and Graves' disease (GD). Although existing evidence suggests increased oxidative damage in patients with HT, a controversy exists in the literature on this issue. Therefore, this study aimed to measure whether a difference exists in the serum levels of 8-OHdG between patients with HT and normal participants and assess the correlation between clinical and laboratory values of 8-OHdG.

Material and Methods

Patients

This is a case-control study, and the sample size was calculated using the following formula from Shin SC's study conducted in 2001:

Considering an additional 15% sample to prevent loss and withdrawal, the sample size was determined to be 28 people in each group.

A total of 48 patients (13 men and 35 women; mean age, 42.12±12.59 years; range, 18-75) with HT were enrolled in this study. Of the 48 consecutive patients with newly diagnosed hypothyroidism seen in the endocrinology clinic, 23 had overt hypothyroidism (OHT), and 25 had subclinical hypothyroidism (SCHT). A total of 35 healthy control participants were included: 10 men and 25 women with a mean age of 43.71±14.26 years (range, 20-72). The control participants were age- and sex-matched with the patient group. OHT was defined as an elevated thyroid-stimulating hormone (TSH, >10 μIU/L) with positive antithyroid peroxidase antibodies (TPO, >40 IU/mL). SCHT was characterized as a TSH level between 4 and 10 μIU/L with anti-TPO antibodies (>40 IU/mL). The thyroid function was considered normal when TSH fell within the normal reference ranges. Major exclusion criteria were as follows: (1) pregnancy and lactation; (2) thyroid disorders or surgery; (3) malignancy, chronic inflammatory conditions, and systemic or organ-specific autoimmune diseases, except HT; (4) presence of acute or chronic infection; (5) immunosuppressive treatment, antithyroid drugs therapy, or medications that may affect the thyroid function such as lithium or iodide; and (6) smokers and other tobacco users. The study protocol was completely approved by the Ethics Committee of Tehran University of Medical Sciences through reference no. IR.TUMS.REC.1395.00108. All participants had provided informed written consent before enrollment in the study. Our study was conducted in accordance with the Helsinki Declaration principles.

Demographic conditions and laboratory findings are shown in Table 1.

Assessment of Thyroid Functions

Peripheral venous blood samples were collected from all participants. The sera were

Table 1. Laboratory findings and demographic properties of all patients with Hashimoto's thyroiditis (subclinical hypothyroidism and overt hypothyroidism) and healthy control participants.

Parameters	Total hypothyroid	Subclinical hypothyroid	Overt hypothyroid	Control
Number	48	25	23	34
Age(years)	42.12±12.59	40.36±12.4	44.04±13.7	43.71±14.26
Sex(F/M)	35/13	20/5	15/8	27/8
TSH(μIU/mL)	24.37±30.8*	6.8±1.33∞	43.47±36.11†‡	1.95±0.93
FT4(ng/dL)	1.10±0.35	1.20±0.31	0.99±0.36	1.13±0.26
Anti-TPO(IU/mL)	321±182.64*	314.52±174.43∞	328.08±194.86‡	6.59±4.62
8-OHdG(ng/mL)	12.86±9.41	15.20±11.87	10.31±4.70	12.79±8.87

F: Females; M: Males; TSH: thyroid stimulating hormone; FT4: Serum free T4; Anti-TPO: antithyroid peroxidase antibody; 8-OHdG: 8-hydroxydeoxyguanosine.
P<0.05; *: Total hypothyroid and control; †: Subclinical hypothyroid and overt hypothyroid; ∞: subclinical hypothyroid and control; ‡: overt hypothyroid and control.

separated from the cells by centrifugation and stored at -20 °C until the time of testing.

The serum concentrations of free thyroxine (FT4) and TSH were determined using an enzyme immunoassay kit (Monobind Inc., Lake Forest, CA92630, USA) and immunoradiometric assay kit (IRMA kit Radim, Pomezia, Rome, Italy), respectively. In addition, enzyme immunoassay was used to quantify serum autoantibodies against TPO (Monobind Inc., Lake Forest, CA92630, USA).

Quantitative Determination of 8-OHdG Concentrations

Levels of 8-OHdG were quantified using the enzyme-linked immunosorbent assay kit (Zellbio GmbH, lonsee, Germany) according to the manufacturer's instructions.

Statistics

After determining whether numeric values were normally distributed, the one-way analysis of variance test was used for comparing means of three or more groups. Moreover, the Mann-Whitney and Kruskal-Wallis tests were used for variables that were not normally distributed. A *p*-value of <0.05 was considered statistically significant. A correlation analysis was performed using Pearson's test. All data analyses were performed using SPSS, version 11.0 (SPSS, Inc, Chicago, IL, USA).

Results

This study aimed to assess 8-OHdG serum levels in patients with HT and healthy participants. A total of 83 individuals (35 normal participants, 23 patients with SCHT, and 25 OHT patients) were evaluated. The mean (standard deviation) age of the participants was 42.84 years (13.34) (range, 20-75), and 62 participants were women (74.69%).

The female-to-male ratio in this study was 2.7. No statistically significant difference in terms of age (p=0.52) and sex (p=0.46) was observed between patients and control participants. In this study, the concentration of TSH was significantly higher in patients with OHT and SCHT than healthy participants. Moreover, the level of TSH was higher in the OHT group (range, 10-134 (about 6-fold)) than that in the SCHT group (range, 4.6-9.4; p<0.001). In contrast, the serum FT4 level was lower in the OHT group (range, 0.5-1.65) than the SCHT (range, 0.6-1.7) and control groups (range, 0.8-1.61); however, this difference was not statistically significant (p>0.06). A significant difference was observed in the anti-TPO antibody levels in OHT (range, 74-934 IU/mL) and SCHT groups (range, 75-561 IU/mL) compared with the control group (range, 0.6-18 IU/mL; p<0.001). However, no significant differences were observed in anti-TPO antibody levels between OHT and SCHT subgroups.

A positive correlation was observed between TSH and high anti-TPO antibody concentrations in patients with HT (r=0.72, p<0.01). Moreover, an inverse negative correlation was observed between FT4 and TSH (r=-0.24, p=0.02).

Serum Levels of 8-OHdG

Figure 1 shows the results of 8-OHdG evaluation. Although the results were not statistically significant ($p>0.05$), the figure shows that the level of 8-OHdG in the sera of patients with SCHT (range, 7.2-52 (15.20±11.87)) was higher than those with OHT (range, 6.4-22.9 (10.31±4.7)) and normal control participants (range, 2.7-43 (12.79±8.87)). Moreover, no significant difference was observed in the serum concentration of 8-OHdG between patient groups and healthy participants.

A correlation analysis revealed no association between the serum levels of 8-OHdG and other clinical parameters such as TSH, FT4, and anti-TPO within the patient groups.

Discussion

HT is an autoimmune inflammatory disorder of the thyroid gland whose exact molecular mechanism remains unclear. Several studies have shown that both cellular and humoral immunity play a role in the pathogenesis of HT. The aberrant activation of resident and circulating T and B cells in tissues (the major cellular components of the adaptive immune response) triggers a cascade of immunological events, leading to increased production of autoantibodies, apoptosis, and alteration of cytokine balance (9).

On the other hand, redox homeostasis is essential for the appropriate functioning of the immune system (10). Whereas sustained imbalances in redox homeostasis play a major role in the etiology of autoimmune disorders (11).

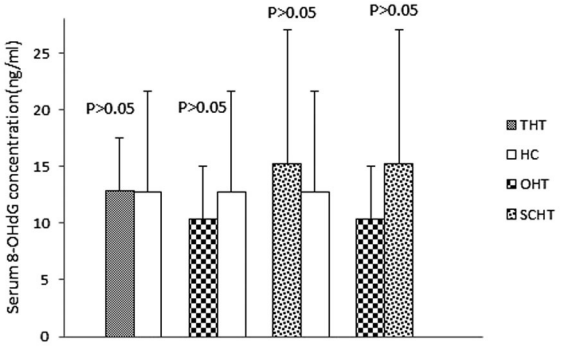

Figure 1. Serum 8-OHdG levels in study groups. Each bar represents the average values of 8-OHdG levels with Standard deviation in the four groups including total hypothyroid (THT), overt hypothyroid (OHT), subclinical hypothyroid (SCHT) patients and healthy controls (HC). There were no statistically significant differences between groups.

It is well known that increased oxidative stress affects organic molecules. The oxidation of these crucial biological molecules was confirmed by the formation of oxidation byproducts that can serve as useful biomarkers in assessing oxidative stress. In recent years, great attention has been focused on oxidative stress-related biomarkers as their accurate evaluation can help in studying their role in disease pathogenesis and monitoring treatment success (12).

Until date, different types of oxidative markers have been identified in the body. 8-OHdG is one of the most crucial, abundant, and sensitive biomarkers of oxidative DNA damage (13). The removal of 8-OHdG is necessary for maintaining genome integrity. After excision, 8-OHdG is exported into the serum, urine, or other extracellular fluids without further metabolism (14-16). Therefore, it can be quantified in body fluid samples.

In this study, the levels of 8-OHdG were measured in the sera of patients with HT and healthy control participants. To the best of our knowledge, this is the first study to explore changes in 8-OHdG levels in the sera of patients with HT. The results showed no significant difference in the concentration of 8-OHdG between patients and control participants, which is in line with the results of some previous studies.

Our findings are in line with some previous literature results, showing no significant changes in the amount of 8-OHdG between patients with HT and controls. For instance, in a study conducted by Hara et al., the production of 8-OHdG and cytochrome c was analyzed in the culture supernatant of mononuclear cells derived from the peripheral blood of patients with GD and HT. The levels of cytochrome c were significantly higher in untreated patients with GD and HT than that in healthy individuals, whereas the 8-OHdG level increased only in patients with GD (17).

In contrast, results from other studies have shown that the 8-OHdG level was significantly increased in patients with HT compared with that in normal control participants. For instance, the overexpression of 8-OHdG has been observed in the thyroid tissues of patients with HT, GD, and

papillary thyroid carcinoma (18). These findings have also been supported by other research evidence indicating an elevated level of 8-OHdG in the urine of patients with toxic multinodular goiter, GD, and HT (19). The discrepancies between these results can partly be associated with several possible explanations particularly related to the patient's treatment status or sites/organs from which the specimens were obtained (20). Therefore, a simultaneous tracing of 8-OHdG in the thyroid and body fluids is essential for a better understanding of its role in HT. Moreover, a comparison of 8-OHdG levels before and after treatment can provide valuable information regarding the role of this indicator in endogenous oxidative DNA damage.

Small sample size, the absence of data regarding 8-OHdG levels in the thyroid tissue of patients with HT, and the lack of any experimental design for assessing treatment-related alterations in the composition of 8-OHdG were three major limitations of this study, which can be addressed in future studies. Moreover, simultaneous measurements of 8-OHdG and other markers of oxidative stress can provide a more precise interpretation and improve the validity and reliability of the data.

Conclusion

This study does not indicate any difference in the levels of 8-OHdG between patients with HT and healthy participants. However, the role of oxidative stress in HT cannot be ruled out from these findings. Therefore, more studies are needed to analyze the potential link between oxidative stress and the pathogenesis of HT.

Financial Support and Sponsorship

This study was conducted with the financial support of the Tehran University of Medical Sciences for Research through contract no. 34052.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Zohreh Jadali, Fatemeh Esfahanian; Design: Zohreh Jadali, Fatemeh Esfahanian; Control/Supervision: Fatemeh Esfahanian; Data Collection and/or Processing: Seyedeh Mahdieh Fotouk Kiaie, Roghayeh Ghelich; Analysis and/or Interpretation: Seyedeh Mahdieh Fotouk Kiaie; Literature Review: Seyedeh Mahdieh Fotouk Kiaie; Writing the Article: Zohreh Jadali; Critical Review: Fatemeh Esfahanian; References and Fundings: Tehran University of Medical Sciences; Materials: Fatemeh Esfahanian, Seyedeh Mahdieh Fotouk Kiaie, Roghayeh Ghelich.

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