Toxicology Mechanisms and Methods

ISSN: 1537-6516 (Print) 1537-6524 (Online) Journal homepage: https://www.tandfonline.com/loi/itxm20

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To cite this article: Unzile Sur, Pinar Erkekoglu, Ayse Derya Bulus, Nesibe Andiran & Belma Kocer-Gumusel (2019) Oxidative stress markers, trace elements, and endocrine disrupting chemicals in children with Hashimoto's thyroiditis, Toxicology Mechanisms and Methods, 29:9, 633-643, DOI: 10.1080/15376516.2019.1646367

To link to this article: https://doi.org/10.1080/15376516.2019.1646367

TOXICOLOGY MECHANISMS AND METHODS
2019, VOL. 29, NO. 9, 633–643
https://doi.org/10.1080/15376516.2019.1646367

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RESEARCH ARTICLE

Oxidative stress markers, trace elements, and endocrine disrupting chemicals in children with Hashimoto's thyroiditis

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ABSTRACT

In this study, we aimed to investigate whether bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP) exposure have any association with Hashimoto's thyroiditis (HT) and its biomarkers and to determine whether oxidative stress biomarkers and trace element levels showed any alterations in children with HT. We found that superoxide dismutase and glutathione peroxidase activities are lower in HT group from control (24% and 46%, respectively, $p < 0.05$). Zinc levels were significantly lower in HT group vs. control. In addition, the levels of mono-(2-ethylhexyl) phthalate (MEHP) which is the primary metabolite for DEHP, were markedly higher in HT group compared to control ($p < 0.05$). A negative correlation was observed between urinary BPA levels and fT4. In children with HT, oxidant/antioxidant balance is changed and these differences may be related by EDC exposure, the importance of which should be elucidated with further studies.

ARTICLE HISTORY
Received 21 March 2019
Revised 11 July 2019
Accepted 14 July 2019

KEYWORDS
Bisphenol A; di-(2-ethylhexyl) phthalate; Hashimoto's thyroiditis; iodine; oxidative stress; selenium; thyroid hormones; zinc

Introduction

Hashimoto's thyroiditis (HT), which is also known as 'autoimmune thyroiditis' or 'chronic lymphocytic thyroiditis', was first characterized by Hakura Hashimoto in 1912. In HT patients, thyroid gland is diffusely enlarged and becomes rigid with distinctive pathologic appearance (Hashimoto 1912). Presence of elevated thyroid peroxidase auto-antibodies (anti-TPOs) is the important biomarker for HT diagnosis. It is well known that 40–50% of HT patients have a family member with thyroid disorder. Incidence is related to ethnicity, age, gender, and environmental factors. Prevalence of HT is 2% in the general population and women are 5- to 10-fold more vulnerable than men. A research on American children and adolescents showed that HT was more common among girls. The most frequent age range is defined as 10–11 years (Chiovato et al. 1993; Vanderpump et al. 1995; Setian 2007). Though thyroid diseases are largely encountered in Turkey, particularly in Black Sea region (Bastemir et al. 2006), there are not any large-scale HT prevalence studies in Turkish population.

In recent years, the results of considerable number of studies have revealed that autoimmune disorders are most commonly seen in massively industrialized regions and it is suggested that exposure to 'endocrine disrupting chemicals (EDCs)' may be one of the underlying factors of autoimmune disorders (Shapira et al. 2010). Plastic hardeners, such as bisphenol A (BPA), and plastic softeners, such as phthalates, are suggested to be the most abundant EDCs in the environment (Ahmed 2000). Although the most widely used phthalate derivative di-(2-ethylhexyl) phthalate (DEHP) and its primary metabolite mono-(2-ethylhexyl) phthalate (MEHP) are not proven to be immunogenic antigens, there are some studies showing they can act as adjuvants (Inoue et al. 2006; Hansen et al. 2007; Larsen et al. 2007). Furthermore, there are a few studies showing a link between phthalates and autoimmunity (Lim and Ghosh 2005; Cooper et al. 2010); however, there is no human or animal data showing an association between phthalates and autoimmune thyroid disorders. On the other hand, it was suggested that both DEHP and MEHP might alter thyroid hormone status in rodents and humans (Meeker et al. 2007; Erkekoglu et al. 2012a; Johns et al. 2015; Liu et al. 2015). In addition, various studies showed that BPA may alter immune processes by affecting macrophages, $CD4^+$ T cells, immunoglobulin (Ig), and interleukin (IL) levels both *in vitro* and in mice (Lee et al. 2003; Tian et al. 2003). It was also demonstrated that BPA could affect thyroid histology, thyroid hormone and TSH levels in rats (Moriyama et al. 2002; Boas et al. 2009).

The interactions of EDCs with thyroid gland may increase oxidative stress or inflammation which could play important role for the pathogenesis of thyroid diseases (Giray et al. 2010; Mostafa et al. 2010; Poncin et al. 2010; Aslan et al. 2011; Žarković 2012). However, there is limited number of studies that underline the importance of oxidant/antioxidant balance in HT and these studies are mostly conducted on adult HT patients (Öztürk et al. 2012; Ates et al. 2015; Ruggeri et al. 2016). Furthermore, trace element status (i.e. iodine, selenium, and zinc) is essentially important for

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both the processing of thyroid hormones and reducing the damaging effects of oxidative stress within the thyroid gland. Trace elements may affect the thyroidal homeostasis by affecting thyroid hormone production and participating in the structure of important enzymes in the thyroid hormone synthesis pathway (Behne et al. 1990; Ganapathy and Volpe 1999; Schomburg and Köhrle 2008; Nourbakhsh et al. 2016).

There are no adequate data on the alterations in oxidant/antioxidant parameters including selenoproteins and trace element status in children with HT (Öztürk et al. 2012; Rostami et al. 2013). There is also lack of human data on the associations between EDCs and autoimmune thyroidal diseases for both adults and children. Concerning all the presented data herein, we aimed to investigate whether BPA and DEHP exposures have any association with HT and its biomarkers. Moreover, we also aimed to determine whether oxidative stress biomarkers showed any alterations in newly diagnosed children with HT. To our knowledge, this is the first study, suggesting an association between trace elements, oxidative stress parameters, EDCs, and HT in children.

Materials and methods

Subjects

The study group consisted of 29 children (age = 8–16 years, three boys, 26 girls) who were admitted to Keçioren Research and Training Hospital, Pediatric Endocrinology Unit between August 2014 and March 2015. Study group was selected from newly diagnosed children with HT. The children were not taking any treatment for HT by then. In addition, these children did not have any other chronic, endocrine or genetic disease. HT diagnosis was made by following criteria: (i) increased plasma levels of anti-TPO; (ii) heterogeneous echo-texture in thyroidal ultrasound.

Control group ($n = 29$, age = 8–16 years, four boys, 25 girls) was selected from age-matched healthy children without any chronic, endocrine or genetic disease. Children in both study and control groups were not taking any regular medication or supplement. A standard questionnaire was applied to parents to determine educational status, occupational information, prenatal medications, familial thyroid diseases or other endocrine diseases, dietary habits, and potential exposure route to EDCs.

The study was approved by Keçioren Research and Training Hospital's Ethical Committee. Written informed consent was obtained from the parents of the children.

Deplasticization of the glassware

Extreme caution was taken for preventing contact with plastic material throughout the study. All the glassware used for the collection of urine samples were deplasticized with tetrahydrofuran:n-hexane (50:50 (v/v)) for 2 h and later dried in an incubator for 2 h at 37 °C. All the test tubes were deplasticized on a heater for 4 h at 400 °C.

Sample preparation

Heparinized blood samples (~10 mL) were taken by a stainless steel needle into deplasticized glass test tubes for plasma phthalate determinations and oxidant/antioxidant parameter measurements. The tube openings were covered by clean aluminum foil to protect the sample from contact with plastic material. Heparin-free blood samples (~5 mL) were taken for the measurement of thyroid hormone parameters and zinc levels. Samples were centrifuged immediately at $800 \times g$ for 15 min in order to separate plasma and erythrocytes. Both plasma and erythrocyte samples were aliquoted and kept at −80 °C until analysis.

Spot urine samples (~5 mL) were collected into deplasticized glass beakers for the determination of urinary BPA levels. The urine samples were aliquoted and kept at −80 °C until analysis.

Chemicals and kits

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) except MEHP which was obtained from Cambridge Chemicals (Woburn, MA). Electrochemiluminescence immunoassay (ECLIA) kits for the determination of fT4, fT3, and TSH were from Diasorin Liaison (Stillwater, MN). Colorimetric assay kits for protein determination, glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) were obtained from Cayman Chemical Company (Ann Arbor, MI). Selenoprotein P (SePP) kit was obtained from East Biopharm Company (Hangzhou, China). All high-performance liquid chromatography (HPLC) equipments were from Agilent (Santa Clara, CA).

Determination of thyroid hormone parameters

Serum thyroid hormone (fT_4, fT_3, and TSH) levels and anti-TPO levels were measured by using 'Liaison DiaSorin CLIA kits' on a Diasorin Liaison CLIA Analyzer (Stillwater, MN). TSH kits were based on third generation two-site CLIA assay with two monoclonal antibodies and analytical sensitivity of 0.004 mIU/L while fT_4 and fT_3 kits employed one-step simultaneous competitive CLIA with SPALT monoclonal isoluminol-labeled antibody. Anti-TPO kit was also based on competitive CLIA method.

Determination of antioxidant parameters

The commercial kit used in the study indirectly measures the activity of GPx by measuring the decrease in absorbance at 340 nm due to oxidation of NADPH to $NADP^+$. GPx activity was expressed as nmol/min/mg hemoglobin (Hb).

The total SOD activity was measured by a commercial kit using colorimetric assay showing the decrease in color development at 440 nm due to inhibition of xanthine oxidase activity by SOD for conversion of xanthine and water to uric acid and hydrogen peroxide (H_2O_2). SOD activity was expressed as U/mg Hb.

The commercial kit which is used for CAT activity was based on the reaction of CAT with methanol in the optimal concentration of H_2O_2. As a result of this reaction, formaldehyde is produced which is directly proportional to CAT activity. The amount of formaldehyde is spectrophotometrically measured in the presence of the chromogen '4-amino-3-hydrazino-5-mercapto-1,2,4-triazole'. CAT activity was expressed as nmol/min/mg Hb.

Hemoglobin levels

Hemoglobin levels in erythrocytes were determined according to Fairbanks and Klee (1986). Optical densities were read at 546 nm spectrophotometrically.

Determination of total glutathione and lipid peroxidation levels

Total GSH levels were measured by using a total GSH assay kit based on an enzymatic recycling method measuring the DTNB-TNB conversion which is directly proportional to GSH concentration in the sample. Measurement of the absorbance was at 405 nm and the results were expressed as nmol/mg Hb.

As an indicator of lipid peroxidation, malondialdehyde (MDA) levels were measured by HPLC (Templar et al. 1999). Briefly, MDA-thiobarbituric acid (TBA) adducts were formed at 100 °C; extracted by n-butanol; diluted and MDA concentrations were measured (Hewlett Packard Agilent 1200 Series with Fluorescence Detector, Vienna, Austria). Throughout the experiments, analytical column was Spherisorb C18 ODS2 column (25 cm × 5 m × 4.6 mm i.d.). Mobile phase was methanol:KH_2PO_4-KOH (pH: 7.0, 65:35 (v/v)). Flow rate was 1 mL/min and UV detector was set at $\lambda_{excitation}$: 515 nm and $\lambda_{emission}$: 550 nm. Injection volume was 100 μL. Plasma MDA concentrations were calculated from standard curve prepared from 1,1,3,3 tetraethoxypropane and expressed as μmol/L. Limit of detection (LOD) was 37.5 nmol/L.

Determination of selenoprotein P levels

The measurement of plasma SePP levels was performed by a kit that employs a quantitative double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of SePP in plasma samples. The concentration of SePP in the samples was calculated from the calibration curve obtained from SePP standards. The intensity of the color was measured at 450 nm and readings at 540 nm were subtracted from the readings at 450 nm. The results were expressed as ng/mL.

Determination of trace element levels

A single-beam atomic absorption spectrometer (PerkinElmer AAS Spectrometer 700, Waltham, MA) with a Zeeman background correction equipped with an Fs-go plus furnace and auto-sampler was used to determination of plasma selenium levels according to a well-known measurement method (Kırkbright 1980). Results are expressed as μg/L.

Urinary iodine levels were measured by iodine-azide method at 366 nm spectrophotometrically. Results are expressed as μg/dL.

Serum zinc levels were measured in Beckmann Coulter AU 680 (Miami, FL) at 560 nm spectrophotometrically. Results are expressed as μg/dL.

Measurement of plasma di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate levels

Plasma DEHP and MEHP levels were detected by HPLC according to Paris et al. (2003) with some modifications. Briefly, plasma (200 μL) was spiked with DEHP and MEHP (1 ppm in the last volume, both). After extraction by NaOH (1 N, 400 μL), 50% H_3PO_4 (100 μL) and acetonitrile (800 μL), samples were vortexed for 1 min. The mixture was centrifuged at 5000 rpm for 10 min. Supernatant (600 μL) was taken into another tube and evaporated under nitrogen stream. The residues were kept at −20 °C until analysis. Later, residues were dissolved in acetonitrile (60% (v/v), 300 μL) and the resultants (100 μL) were injected to HPLC (Hewlett Packard Agilent 1200 Series, Vienna, Austria; equipped with an auto sampler and a UV detector). Spherisorb C18 ODS2 column (25 cm × 5 m × 4.6 mm i.d.) (Waters, Milford, MA) and ODS C18 precolumn (4 cm) (Waters, Milford, MA) were used for analysis. The mobile phase was 0.1% phosphoric acid and acetonitrile (pH 3.0, 20:80 (v/v)), and the flow rate was 1 mL/min. The retention times for DEHP and MEHP were 39.3 min and 4.7 min, respectively. The concentrations of DEHP and MEHP in the samples were calculated from DEHP and MEHP standards and the calibration curve of peak area was used. LODs for both DEHP and MEHP were 0.05 g/mL.

Measurement of urinary bisphenol A levels

For the analysis of urinary BPA, the method of Yang et al. (2003) was used with some modifications. Briefly, after spiking urine with BPA (5 ng/mL in the last volume), sodium acetate buffer (2.0 M, 30 μL, pH: 5), and β-glucuronidase/arylsulfatase (10 μL, from Helix pomatia) were added on this mixture and vortexed. The mixture was incubated at 37 °C for 3 h in order to free the conjugated BPA (glucuronidated + sulfated). After incubation, HCl (100 μL, 2 N) and ethyl acetate (5 mL) were added to the mixture and centrifuged. Supernatant (3 mL) was evaporated under nitrogen stream; the glass tubes with residues were kept at −20 °C until analysis. Later, the residues were dissolved in acetonitrile (300 μL, 60%) and samples (100 μL) were injected to HPLC (Hewlett-Packard Agilent 1100 series, Santa Clara, CA; equipped with an auto sampler and a fluorescence detector). HPLC parameters were as follows: C18 column (25 cm × 5 μm × 4.6 mm i.d.); column temperature: 25 °C; $\lambda_{excitation} = 230$ nm and $\lambda_{emission} = 315$ nm. Mobile phase was acetonitrile:tetrahydrofuran (2.5% (v/v)) and gradient elution was applied as 60:40 to 5:95). Flow rate was 0.4 mL/min and retention time was 18.3–19.2 min. LOD was 0.5 ng/mL. The concentration of BPA in the samples was calculated from

BPA standards and the calibration curve of peak area was used.

Urinary creatinine concentrations were analyzed simultaneously by HPLC according to Jen et al. (2002) with slight modifications and the urinary BPA concentrations were adjusted by urinary creatinine concentrations.

Statistical analysis

Statistical analysis was performed by using SPSS 22.0 (SPSS Inc., Chicago, IL). The distribution of values was analyzed by the Levene test. The comparison between two parametric values was determined by using Student's *t*-test. For non-parametric comparisons, the Mann–Whitney *U*-test was used. Categorical variables were compared by using chi-square test. The correlation between values was analyzed by using Spearman's correlation or Pearson's correlation according to being parametric or non-parametric. Data were expressed as mean ± SEM. p Values <0.05 were considered as statistically significant.

Results

Characteristics of study population

The study and control groups were similar in terms of age, sex, height, weight, and relative body weight. Basic characteristics of the patients and healthy controls are shown in Table 1. There was no significant difference between groups concerning these parameters ($p > 0.05$, all).

Questionnaire evaluations

None of the subjects had prenatal exposure to drugs. Familial hormonal disease frequency (including thyroid diseases) was significantly higher in the study group than the control group ($p < 0.05$). Although the frequency of endocrine diseases (except thyroidal diseases) between the first degree relatives (mother, father, siblings) was not significantly different between the groups ($p > 0.05$), thyroidal disease frequency in study group was markedly higher than the control group ($p < 0.01$) (Table 2). On the other hand, plastic bottle and cosmetic use, playing with plastic toys and ready-made food consumption of the two groups were not significantly different ($p > 0.05$).

Table 1. Basic characteristics of the study groups.

Characteristics	Control ($n = 29$)	HT ($n = 29$)
Gender	3 boys, 26 girls	4 boys, 25 girls
Age (years)	13.02 ± 0.45	12.97 ± 0.45
Height (cm)	150.31 ± 2.18	153.23 ± 2.32
Weight (kg)	43.72 ± 1.86	48.22 ± 2.55
Relative body weight	98.93 ± 3.46	108.29 ± 4.19
BMI	19.19 ± 0.68	20.24 ± 0.69

BMI: body mass index; HT: Hashimoto's thyroiditis.
All values are shown as mean ± SEM.

Thyroid hormones and anti-TPO levels

TSH, fT_3, fT_4, and anti-TPO levels of the study groups are shown in Figure 1. TSH and anti-TPO levels (460-fold) were significantly higher when compared to control. On the other hand, fT_3 and fT_4 levels were significantly lower in HT group vs. control ($p < 0.001$).

Trace element levels

Urinary iodine, plasma selenium, and serum zinc levels are shown in Figure 2. Although there were no significant difference in selenium levels ($p > 0.05$), iodine and zinc levels were significantly lower in HT group vs. control ($p < 0.001$). In HT group, 38% of the children ($n = 11$) had normal urinary iodine levels (10–16.7 μg/dL) while 59% of children ($n = 17$) had mild iodine deficiency (5.2–9.6 μg/dL) and 3% of the children ($n = 1$) had moderate iodine deficiency (3.8 μg/dL).

Oxidant/antioxidant status and selenoprotein P levels

Plasma MDA, total GSH, SOD, CAT, GPx, and SePP levels in the study groups are shown in Table 3. There were no significant differences in MDA, SePP, and CAT activity between the groups ($p > 0.05$, all). Though total GSH levels decreased in HT group (23.6%), it was not statistically significant vs. control ($p = 0.07$). In addition, SOD and GPx activity significantly decreased in HT group when compared to control group ($p < 0.05$).

Bisphenol A, di-(2-ethylhexyl) phthalate, and mono-(2-ethylhexyl) phthalate levels

Plasma DEHP and MEHP levels and urinary BPA levels are shown in Table 4. There was 21.5% increase in plasma DEHP levels in HT group vs. control ($p > 0.05$). Plasma MEHP levels showed 138.2% increase and this elevation was significant vs. control ($p = 0.01$). Urinary BPA levels were not also significantly different in HT group vs. control. Additionally, a high percentage of the patients (58.8%) who never used teething ring had lower urinary BPA levels than the ones who used teething ring in their childhood ($p = 0.05$). While 58.3% of subjects who regularly consumed fast food had higher BPA levels, 85.7% of the patients who never consumed fast food had lower BPA levels ($p = 0.019$). However, there was no

Table 2. Questionnaire evaluations for the study groups.

	Control		HT		
	n	%	*n*	%	*p*
Familial hormonal diseases					
Yes	2	6.9	8	27.6	$p < 0.05$
No	27	93.21	21	72.4	
Thyroidal diseases in first degree relative					
Yes	6	20.7	17	58.6	$p < 0.01$
No	23	79.3	12	41.4	
Endocrine diseases in first degree relative (except thyroidal)					
Yes	6	20.7	9	31	$p > 0.05$
No	23	79.3	20	69	

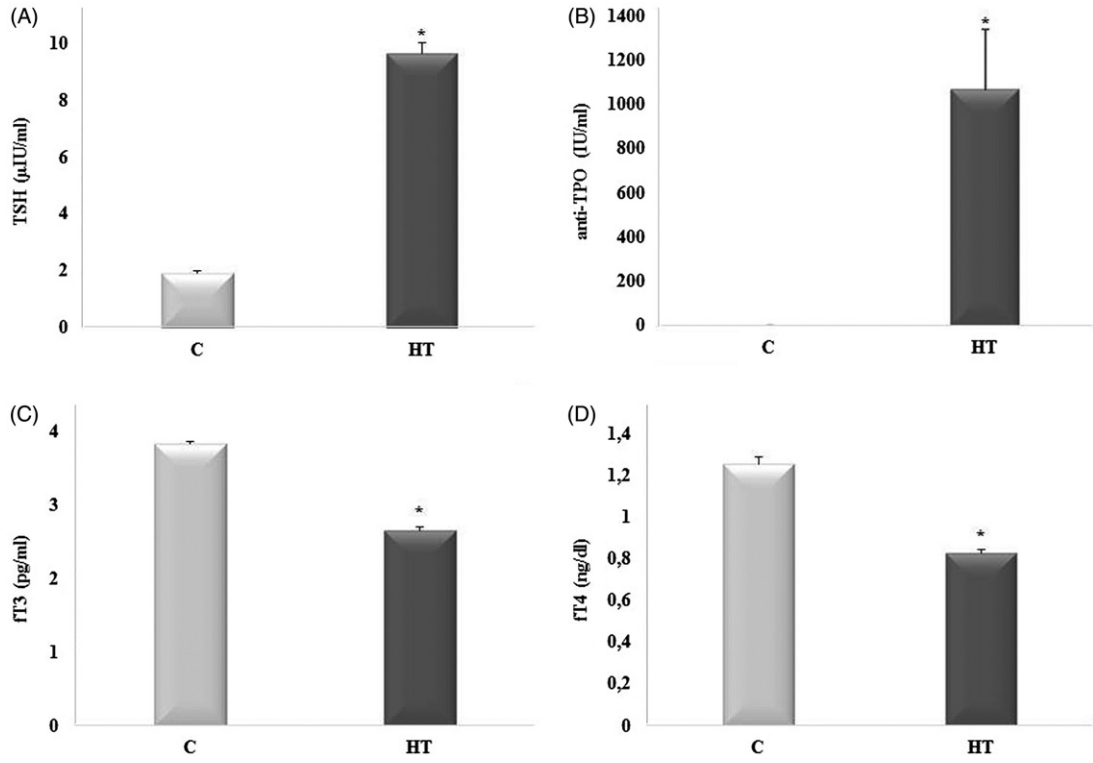

Figure 1. Serum TSH, fT3, fT4, and anti-TPO levels in the study groups. (A) TSH levels, (B) anti-TPO levels, (C) fT3 levels, (D) fT4 levels anti-TPO: anti thyroid peroxidase; fT_3: free triiodothyronine; fT_4: free thyroxine; TSH: thyroid stimulating hormone. $^*p < 0.001$ vs. control.

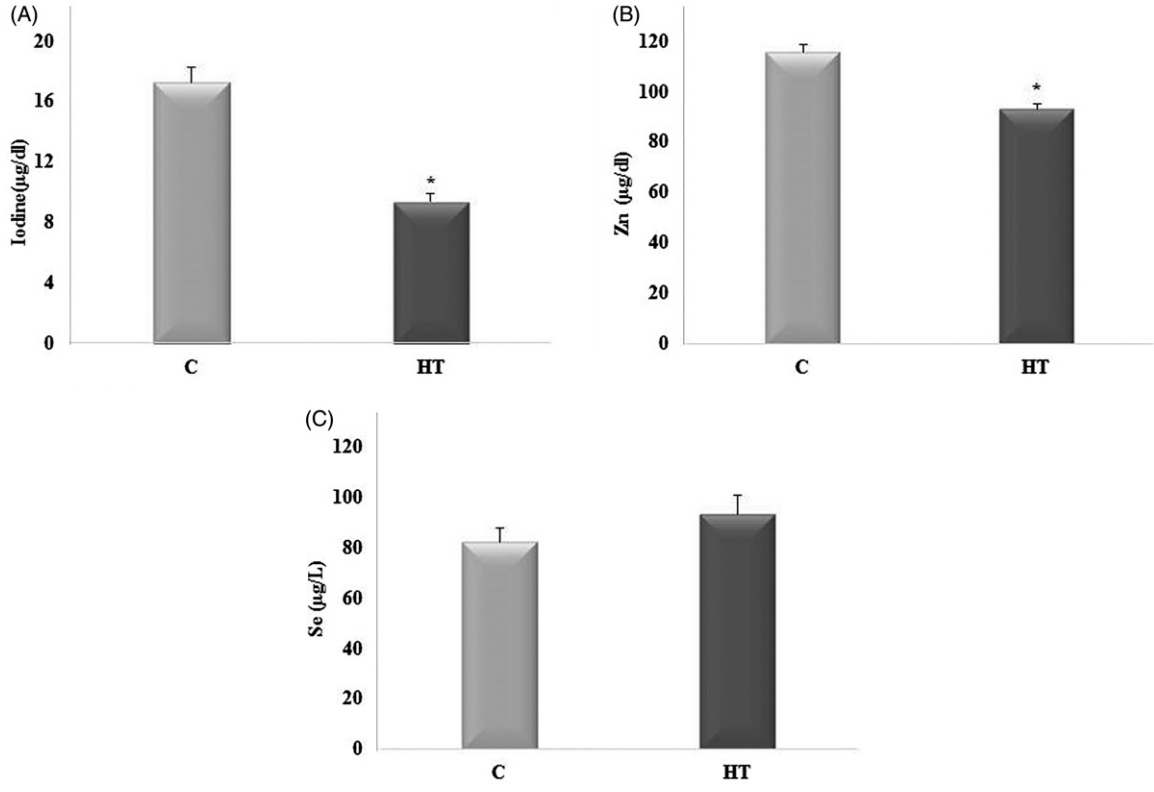

Figure 2. Urinary iodine, plasma selenium and serum zinc levels in the study groups. (A) Urinary iodine levels, (B) serum zinc levels, and (C) plasma selenium levels. Se: selenium; Zn: zinc. $^*p < 0.001$ vs. control.

significant association between BPA, DEHP, and MEHP levels and plastic exposure from other sources (plastic bottles, consumer products, residence in industrial area, etc.) (data not shown).

Correlations between the measured parameters

A significant positive correlation was found between fT_3 and GSH ($r = 0.400$, $p < 0.05$) as well as between fT_3 and SePP

Table 3. Plasma MDA, total GSH, SOD, CAT, GPx, and selenoprotein P (SePP) levels in study groups.

	SOD (U/mg Hb)	CAT (nmol/min/mg Hb)	GPx (nmol/min/mg Hb)	GSH (nmol/mg Hb)	MDA (μmol/L)	SePP (ng/mL)
Control ($n = 29$)	4.27 ± 0.42	318.98 ± 27.08	29.13 ± 3.85	25.83 ± 2.65	1.36 ± 0.07	76.20 ± 6.58
HT ($n = 29$)	3.24 ± 0.19[a]	331.78 ± 19.82	15.89 ± 1.71[a]	19.74 ± 2.00	1.30 ± 0.06	65.17 ± 5.78

CAT: catalase; GPx: glutathione peroxidase; GSH: glutathione; HT: Hashimoto's thyroiditis; MDA: malondialdehyde; SePP: selenoprotein P; SOD: superoxide dismutase.
All results were shown as mean ± SEM.
[a]$p < 0.05$ vs. control.

Table 4. Plasma DEHP and MEHP and urinary BPA levels in the study groups.

	DEHP (μg/mL)	MEHP (μg/mL)	BPA (μg/g creatinine)
Control ($n = 29$)	0.46 ± 0.74	0.20 ± 0.48	7.72 ± 1.74
HT ($n = 29$)	0.56 ± 0.66	0.48 ± 0.61*	7.31 ± 1.46

BPA: bisphenol A; DEHP: di-(2-ethylhexyl) phthalate; HT: Hashimoto's thyroiditis; MEHP: mono-(2-ethylhexyl) phthalate.
All results were shown as mean ± SEM.
*$p = 0.01$ vs. control.

Table 5. Correlations between thyroid hormones and other parameters in HT group.

	Correlation coefficient (r)
TSH	
GSH	–0.366 ($p = 0.056$)
GPx	–0.442[a]
fT_3	
GSH	0.400[a]
SePP	0.442[b]
fT_4	
CAT	0.358 ($p = 0.056$)
Selenium	–0.350 ($p = 0.063$)
BPA	–0.483[b]
Anti-TPO	
Iodine	–0.404[a]

Anti-TPO: anti-thyroid peroxidase; BPA: bisphenol A; CAT: catalase; fT_3: free triiodothyronine; fT_4: free thyroxine; GPx: glutathione peroxidase; GSH: glutathione; SePP: selenoprotein P; TSH: thyroid stimulating hormone.
[a]$p < 0.05$.
[b]$p < 0.02$.

($r = 0.442$, $p < 0.02$). There were significant negative correlations between TSH and GPx1 (r=–0.442, $p < 0.05$), anti-TPO and iodine (r=–0.404, $p < 0.05$) and fT_4 and BPA (r=–0.483, $p < 0.02$). Although, there were negative relations between TSH and GSH and fT4 and selenium and a positive relation between fT_4 and CAT, these relations were not statistically significant possibly due to the low subject number within the HT group (r=–0.366, $p = 0.056$; r=–0.350, $p = 0.063$ and $r = 0.358$, $p = 0.056$, respectively) (Table 5).

We have also evaluated the correlations between trace element levels and other parameters (Table 6). According to the results, there was a negative correlation between anti-TPO and iodine levels (r=–0.404, $p < 0.05$). Although there was a positive association between zinc and Se levels, this relation was not statistically significant, again possibly due to low number of subjects within the HT group ($r = 0.343$, $p = 0.068$). Furthermore, selenium had a significant negative correlation with antioxidant enzyme CAT (r=–0.371, $p < 0.05$). Selenium also had an insignificant negative correlation with fT4 (r=–0.350, $p = 0.063$).

The correlations between oxidant/antioxidant status, SePP levels with other measured parameters and the correlations are given in Table 7. SOD and CAT and CAT and fT_4 had positive correlations; however, both were not significant ($r = 0.363$, $p = 0.058$ and $r = 0.358$, $p = 0.056$, respectively). In addition,

Table 6. Correlations between trace element levels and other parameters in the HT group.

	Correlation coefficient (r)
Iodine	
Anti-TPO	–0.404[a]
Zinc	
Selenium	0.343 ($p = 0.068$)
Selenium	
Zinc	0.343 ($p = 0.068$)
CAT	–0.371[a]
fT_4	–0.350 ($p = 0.063$)

Anti-TPO: anti-thyroid peroxidase; CAT: catalase; fT_4: free thyroxine; HT: Hashimoto's thyroiditis.
[a]$p < 0.05$.

Table 7. Correlations between oxidant/antioxidant parameters and other measured parameters in the HT group.

	Correlation coefficient
SOD	
CAT	0.363 ($p = 0.058$)
CAT	
SOD	0.363 ($p = 0.058$)
SePP	0.377[a]
Selenium	–0.371[a]
Zinc	–0.407b
SePP	
MEHP	0.355 ($p = 0.064$)

CAT: catalase; GSH: glutathione; HT: Hashimoto's thyroiditis; MEHP: mono-(2-ethylhexyl) phthalate; SePP: selenoprotein P; SOD: superoxide dismutase.
[a]$p < 0.05$.
[b]$p < 0.02$.

CAT was positively and significantly correlated with SePP ($r = 0.377$, $p < 0.05$) and negatively and markedly correlated with selenium and zinc (r=–0.371, $p < 0.05$ and r=–0.407, $p < 0.02$, respectively). The antioxidant enzyme GPx3 was positively correlated with SePP ($r = 0.408$, $p < 0.05$) and MEHP ($r = 0.390$, $p < 0.05$). Moreover, there was an insignificant association between SePP and MEHP ($r = 0.355$, $p = 0.064$).

Discussion

Hashimoto's thyroiditis is an autoimmune disease which is generally encountered in adulthood, prevalence in children has increased in the last decade. Prevalence between girls at puberty is estimated to be 0.8–1.6%. The present work recruited 25 female (86%) and four male (14%) children with HT diagnosis. The results of the current study can be discussed in five different parts.

Genetic susceptibility

Genetic susceptibility is important for HT development. HT can be diagnosed among the different members of a family

(Huang et al. 2012). In the current work, study subjects were questioned about the presence of familial thyroid diseases and the incidence of other endocrine diseases in first degree relatives. Almost one-third of the children in the HT group had an endocrine disease in their families and thyroid disease was presented in first degree relatives in ~60% of the HT patients.

Thyroid hormones and anti-TPO levels

The TSH level in HT group was ~5-fold higher than control. Both fT_3 and fT_4 levels were significantly lower in HT patients vs. control. These findings indicated that the children in the HT group had a clinical presentation similar to subclinical hypothyroidism. Presence of anti-TPOs was considered as the diagnosis criteria for HT. Anti-TPO levels were almost 460-fold higher in HT patients when compared to control subjects, as expected. Heterogeneity of the thyroid tissue of all HT patients was also observed in the evaluation by ultrasonography. These findings are compatible with the findings of several studies in literature (Korzeniowska et al. 2013; Kahaly et al. 2016; Radetti et al. 2017).

Oxidative stress

During the formation of thyroid hormones, H_2O_2 is continuously used along with tyrosine as a substrate of thyroperoxidase. Therefore, thyrocytes require a very strong antioxidant defense system for maintaining normal thyroid function and protecting the thyroid gland (Degroot and Niepomniszcze 1977). In some cases such as iodine deficiency, higher amounts of TSH may stimulate excess amounts of H_2O_2 production (Degroot and Niepomniszcze 1977). As a result, thyroid tissue may be exposed highly reactive peroxides. Consequently, the presence of a strong antioxidant defense system is important for the removal of oxidative stress (Goyens et al. 1987). The imbalance between oxidant/antioxidant status plays important roles in the pathogenesis of many conditions, including autoimmune thyroid diseases (Žarković 2012; Vitale et al. 2013). Studies conducted in adults have emphasized the role of oxidant/antioxidant status in HT pathogenesis (Öztürk et al. 2012; Rostami et al. 2013); however, there is only one study in literature in children with HT and this study only mentioned the alterations in the activities of paraoxonase and arylesterase (Erol et al. 2016). There are different reports that indicated oxidative stress parameters increased or remained unchanged in patients with hypothyroidism or subclinical hypothyroidism while some studies proposed a decrease in oxidative stress due to the deceleration of metabolic processes (Giray et al. 2001; Torun et al. 2009; Öztürk et al. 2012; Baser et al. 2015). These contradictory results may be observed by reason of age range of study population, gender distribution, having medical treatment or being newly diagnosed. Reddy et al. (2013) suggested that antioxidant defense system in hypothyroid patients was disturbed and decreases in SOD activity and GSH levels were observed in hypothyroid subjects, in accordance with our findings.

It has been reported that increased GPx activity is associated with high TSH levels and stimulation of TSH receptors (Gerenova and Gadjeva 2007). At high amounts, TSH increases the formation of H_2O_2, and consequently high levels of ROS can induce oxidative stress, which has effects on membrane lipids, proteins, and DNA, resulting in necrosis or apoptosis. In the current study, we observed lower GPx activity in HT patients vs. controls; however, no changes were observed in lipid peroxidation. These contradictory results suggest that reduction in GPx activity may be compensated by other cellular mechanisms and may not directly cause lipid damage in cellular membranes. We may suggest that lower GPx activity might may lead to the alterations in thyroid cells. However, whether damage to thyroid cells may lead to the onset of the autoimmune processes is still controversial. Another study performed in adult HT patients showed a significant decrease in GSH levels and 19% increase in serum GPx activity. They stated that this could be an adaptive response to high levels of H_2O_2. The correlation found between anti-TPO levels and GSH levels supported the role of GSH concentrations in the pathology of HT (Rostami et al. 2013). There are also experimental and clinical studies supporting that GSH consumption is involved in the pathogenesis of autoimmune diseases, through the inhibition of IL-1 and T cell receptor mediated signaling mechanisms (Wu et al. 2001; Haq et al. 2007; Ghoreschi et al. 2011). We have also observed 24% decreases in total GSH levels in children with HT. Since GSH is suggested to be a realistic indicator of total body antioxidant capacity, the decrease in GSH levels may be an important marker for the development of oxidative stress in HT. The overall changes in oxidative profile of the study group may be responsible for inflammatory conditions and hormonal deficiency.

Selenium, iodine, and zinc

Iodine and selenium are essential trace elements for thyroidal tissue. Although the studies are rare, zinc also has importance on the conversation of thyroidal homeostasis (Ertek et al. 2010).

As a key element in the structure of thyroidal enzymes, selenium is suggested to regulate inflammatory and immunological response, specifically by increasing GPx and TrxR activities and decreasing H_2O_2 levels in thyroidal tissue (Przybylik-Mazurek et al. 2011). In some studies, researchers reported that anti-TPO and anti-thyroglobulin (anti-Tg) levels were decreased in selenium-supplemented HT patients, suggesting the protective role of selenium against HT (Gärtner et al. 2002; Duntas 2006; Turker et al. 2006). However, other studies showed no significant alterations after selenium supplementation in countries where iodine uptake levels were sufficient (Przybylik-Mazurek et al. 2011; Onal et al. 2012). In autoimmune diseases, including HT, Se supplementation may regulate inflammatory and immunological responses by increasing GPx and TrxR activities, reducing toxic H_2O_2 levels and inflammatory activities. There is evidence from observational studies and randomized controlled trials that selenium supplementation or increased levels/activities of selenoproteins may provide reduction in anti-TPO titers, hypothyroidism,

and postpartum thyroiditis (Hu and Rayman 2017). Although, Se supplementation may be regarded as a subsidiary therapy approach and may be protective/preventive in disease formation, it is not a treatment alternative for HT (Köhrle and Gärtner 2009). In the current study, plasma selenium levels were not significantly different and plasma SePP levels showed a decrease (14%) in HT patients vs. control. On the other hand, there was a statistically significant positive correlation between SePP and sT_3 levels in the HT group ($r = 0.44$, $p < 0.02$), supporting the substantial role of selenium in HT patients for thyroid homeostasis. In addition, although not statistically significant ($p = 0.063$), a correlation was presented between selenium and sT_4 levels in the HT group which also indicates the importance of selenium for thyroid.

Both low and high intakes of iodine may lead to thyroid problems by causing goiter, hyperthyroidism, and hypothyroidism which may soon trigger autoimmunity. The association between high iodine exposure and increased incidence of autoimmune thyroiditis has also been demonstrated in a variety of animal models with genetic susceptibility (Bagchi et al. 1995; Teng et al. 2009; Vecchiatti et al. 2013). In addition, the detrimental effects of iodine deficiency have being elucidated by several epidemiological studies and it has long been a substantial public health issue throughout the world (Pearce et al. 2013). In different studies performed in many countries, there was a relationship between the incidence of HT (Aghini Lombardi et al. 2013), anti-TPO (Gołkowski et al. 2007), and anti-Tg (Premawardhana et al. 2000) with iodine prophylaxis. In the current study, the presence of iodine deficiency in a total of 62% of children in the HT group suggests a possible relationship between iodine deficiency and HT. Oxidative stress observed in iodine deficiency may be considered as a contributing factor to HT formation. As the one of the important parameters for the diagnosis of HT, anti-TPO was also inversely and significantly correlated with urinary iodine levels in HT group ($p < 0.05$).

It has been reported that zinc is also important in normal thyroid homeostasis; however, it has a complex role. Researchers emphasize the importance of the relationship between thyroid gland functions and zinc levels; suggesting that this relationship cannot just be explained by the antioxidant effect of the element and that more extensive studies are required (Ertek et al. 2010). It is reported that the mean plasma/serum zinc levels are in the range of 60–130 μg/dL in healthy individuals (Kolb and Kamyshnikov 1982). In the presented study, we observed that children with HT had significantly decreased (19.33%) serum zinc levels (9.21 μg/dL) vs. control (115.55 μg/dL). Although both group had normal serum zinc levels, HT patients had a tendency for decreased serum zinc concentrations. This effect may be a consequence of disturbance in zinc metabolism or deterioration in zinc transport proteins as a consequence of altered thyroid functions in HT patients. On the other hand, a possible reduction in serum zinc levels may also contribute to the development of HT formation or associated thyroid dysfunction. It is not possible to fully explain the cause–effect relationship with existing findings. There is a need for more mechanistic studies to highlight the association between HT and zinc.

Endocrine disrupting chemicals

Considering the excessive use of chemicals in industrialized populations, exposure to plasticizers such as DEHP, MEHP, or BPA become more important since they have endocrine disruptive properties. A study performed in Taiwan showed that both children who were exposed to high and low molecular weight phthalates had significantly lower serum thyroid stimulating hormone (TSH) levels (Wu et al. 2013). On the other hand, it was suggested that urinary phthalate metabolites may be associated with altered maternal serum thyroid and sex hormone levels and the magnitude of these effects may depend on the timing of exposure during gestation (Johns et al. 2015). In the current study, we did not observe any significant change in plasma DEHP levels in HT patients vs. control. However, MEHP levels were markedly higher in HT patients. This result suggests that the biotransformation of DEHP to MEHP may be increased in HT patients and as it is stated by several studies before, MEHP is responsible for most of the toxic effects of DEHP (Erkekoglu et al. 2010; Ferguson et al. 2014; Zota et al. 2014; Kondolot et al. 2016). However, no significant correlation was observed between plasma phthalate concentrations and thyroid hormones. In addition, we observed that MEHP was positively correlated to SePP levels in HT patients in the current study. This suggests phthalates also interfere with selenium and selenoproteins and this phenomenon was also indicated in our previous studies (Erkekoglu et al. 2012b, 2014).

According to the results obtained from our questionnaire, patients who did not use teething ring in infancy frequently, had low BPA levels. In addition, 58.3% of ready-made food consumers had higher BPA levels than the non-consumers and 85.7% of non-consumers had significantly lower BPA levels than other subjects recruited to this study ($p = 0.0019$). However, there was no significant difference between the urinary BPA levels of HT patients and controls. It is suggested that BPA may directly or indirectly trigger thyroid autoimmunity (Kharrazian 2014). In a study performed on 2361 adult Thai HT patients, the researchers observed association between anti-TPO and serum BPA levels (Chailurkit et al. 2016). In addition, it has been shown that BPA acts as a thyroid receptor antagonist (Zoeller et al. 2005). There is also controversial information about effects of BPA on fT_4 levels (Sriphrapradang et al. 2013; Park et al. 2017). In this present study, there was a negative correlation between BPA levels and fT_4 levels and no correlation was observed between urinary BPA concentrations and anti-TPO levels ($p = 0.063$). As BPA has a similar chemical structure to T_3 and T_4, it may have different effects on thyroid, the importance of which should be further elucidated by mechanistic studies in future.

Conclusions

To our knowledge, this is the first study to examine in detail the relationship between HT, oxidative stress, trace elements, and EDCs. In addition, the fact that the study population consists of children differentiated this study from adult studies.

In conclusion, all these findings show that oxidant/antioxidant balance is impaired in children with HT. However, it is unclear whether oxidative stress is a contributing factor or an outcome in HT. In addition, both iodine and zinc deficiencies can be important contributing factors to the emerging of HT. More extensive work is needed to develop preventive antioxidant approaches for HT and its further complications. On the other hand, determination of other risk factors, such as EDCs that may contribute to HT formation, will provide further information on the formation and prevention of this disease. Further case control studies or cross-sectional studies are needed to elucidate the role of EDCs on autoimmune thyroid diseases.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The entire work was financially supported by the Academic Member Training Program (OYP) of The Council of Higher Education (YOK) of Turkey.

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