

ALTERATION IN PROOXIDANT-ANTIOXIDANT BALANCE ASSOCIATED WITH SELENIUM CONCENTRATION IN PATIENTS WITH CONGENITAL HYPOTHYROIDISM

IZMENE U PROOKSIDANTNOM-ANTIOKSIDANTNOM BALANSU POVEZANE S KONCENTRACIJOM SELENA KOD PACIJENATA SA KONGENITALNOM HIPOTIREOZOM

Shilan Rostami¹, Asadollah Fathollahpour², Mohammad Abdi^{3,4}, Kejal Naderi⁵

¹Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

²Department of Pediatrics, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

³Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

⁴Department of Clinical Biochemistry, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

⁵Food and drugs control laboratory, Vice-chancellery for food and drugs, Kurdistan University of Medical Sciences, Sanandaj, Iran

Summary

Background: There is a paucity of studies regarding oxidative balance and selenium (Se) status in congenital hypothyroidism. Recently, more attention has been given to the use of Se supplementation as a new treatment for thyroid disorders. Oxidative stress increases in different thyroid disorders and causes many pathological outcomes. The present study aimed to determine the serum prooxidant-antioxidant balance (PAB), Se, thyroid stimulating hormone (TSH) and free thyroxine (FT4) concentration in congenital hypothyroidism (CH) patients and the association of their probable change with hematological indices.

Methods: Blood samples were collected from 60 healthy and 39 CH subjects. Serum PAB values were measured and Se, TSH, FT4 and hematological indices were determined. Data were analyzed by SPSS version 16 and *p* value less than 0.05 was considered statistically significant.

Results: Serum TSH concentration was significantly higher in CH patients group compared to the controls (*p* value < 0.05); however, FT4 was in the same concentration in patients and controls. Platelet (Plt) and lymphocytes (Lym) counts markedly decreased in all patients and showed a significant direct correlation with serum TSH levels ($r_s =$

Kratak sadržaj

Uvod: Postoji vrlo malo studija na temu oksidativnog balansa i statusa selena (Se) u kongenitalnoj hipotireozu. Odnedavno se više pažnje pridaje upotrebi suplementacije Se kao novoj terapiji za poremećaje štitne žlezde. Oksidativni stres je povišen u različitim vrstama poremećaja štitne žlezde i izaziva brojne patološke ishode. Ova studija imala je za cilj utvrđivanje prooksidantnog-antioksidantnog balansa (PAB), koncentracija Se, tireostimulišućeg hormona (TSH) i slobodnog tiroksina (FT4) u serumu kod pacijenata sa kongenitalnom hipotireozom (CH) i povezanosti njihovih verovatnih promena sa hematološkim indeksima.

Metode: Uzorci krvi su sakupljeni od 60 zdravih i 39 subjekata sa CH. Vrednosti PAB u serumu su izmerene, dok su Se, TSH, FT4 i hematološki indeksi određeni. Podaci su analizirani pomoću SPSS verzije 16 a *p* vrednost manja od 0,05 je smatrana statistički značajnom.

Rezultati: Koncentracija TSH u serumu bila je značajno viša u grupi pacijenata sa CH u poređenju s kontrolom (*p* vrednost < 0,05); međutim, FT4 bio je u istoj koncentraciji kod pacijenata i kontrolnih subjekata. Ukupni trombociti (Plt) i limfociti (Lym) bili su značajno niži kod svih pacijenata i pokazali su značajnu direktnu korelaciju s nivoima TSH u

Address for correspondence:

Mohammad Abdi
Department of Clinical Biochemistry (room No. 384),
Faculty of Medicine, Kurdistan University of Medical
Sciences, Pasdaran Boulevard, Sanandaj, Iran,
Postal code: 6618634683
Tel: +98-87-33664674 extension 8361,
Fax: +98-87-33664674
e-mail: abdi@muk.ac.ir

0.307, p value = 0.004 and $r_s = 0.413$, p value < 0.0001). Serum PAB and Se had no significant correlation with hematological indices in the studied subjects.

Conclusions: Although there was no correlation between PAB and also Se with hematologic and biochemical markers in CH patients, changes in these two factors might be considered as a potential risk factor due to the other known effects of high PAB values and low Se concentrations.

Keywords: congenital hypothyroidism, free thyroxine, prooxidant-antioxidant balance, selenium, thyroid stimulating hormone

Introduction

Thyroid hormones have a significant effect on modifying the oxidant-antioxidant balance. Alterations in thyroid secretions increase the production of reactive oxygen species (ROS). In the case of congenital hypothyroidism (CH), neurodegenerative complications are highly associated with oxidative stress. Apart from genetic and congenital defects leading to CH, transient CH is considered as the most common pediatric endocrine disorder (1) and can be caused by maternal thyrotropin receptor-blocking antibodies (TRBAs), exposure to maternal antithyroid medications, genetic mutations (such as dual oxidase 2 mutations), untreated hyperthyroidism and iodine deficiency or overload (2–4). In Iran, transient CH included about 80% of primary CH and it has been found that 1 in 294 live births had transient CH (5).

In addition, alteration of selenium (Se) concentration in thyroid disorders has been considered in recent years; however, the possible association between Se concentration and congenital hypothyroidism remains unclear. In addition to the role of this element in the synthesis of thyroid hormones, Se is an important factor in the antioxidant defense against hydrogen peroxide (H_2O_2) molecules. Furthermore, some studies show that Se supplements are very useful for improving the symptoms of thyroid disease. Besides, it has been revealed that oxidative damage to platelets and blood cells correlates with thromboembolism, recurrent infections, and chronic anemia. Selenium has a crucial function in the proper activity of glutathione peroxidases and thus, a disturbance in Se concentration might compromise the GSH content and have a damaging effect on CNS function (6). The decreased GSH content is known as the first trigger for oxidative stress and increase of CNS injury (7). On the other hand, some evidence showed alteration of hematologic indices in thyroid disorders (8–10). However, the role of thyroid hormones on hematologic indices is still controversial. Our previous study showed that higher oxidant levels link to changes in hematologic indices like white blood cells (WBC) count, red blood cells (RBC) count and hemoglobin (Hb) concentration (11, 12). Concerning the hypothyroid patients, some studies showed that total leucocyte, neutrophils and platelets count may be slightly

serumu ($r_s = 0,307$, p vrednost = 0,004 i $r_s = 0,413$, p vrednost < 0,0001). PAB i Se u serumu nisu bili u značajnoj korelaciji sa hematološkim indeksima kod ispitivanih subjekata.

Zaključak: Iako nije bilo korelacije između PAB niti Se i hematoloških i biohemijskih markera kod obolelih od CH, promene ova dva faktora mogle bi se smatrati za potencijalni faktor rizika zbog drugih poznatih efekata visoke vrednosti PAB i niske koncentracije Se.

Ključne reči: kongenitalna hipotireoza, slobodni tiroksin, prooksidantni-antioksidantni balans, selen, tireostimulišući hormon

decreased (13). Besides, it was previously shown that hypothyroidism-induced anemia, which is characterized by low RBC count, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), hemoglobin (Hb) and hematocrit (Hct) (8) or hyperproliferation of immature erythroid progenitors, is not related to thyroid hormones (10).

According to the above-mentioned, hematological changes in CH are still controversial and to the best of our knowledge, there is no other study about the association of Se concentration with prooxidant-antioxidant balance (PAB) and hematological parameters in congenital hypothyroidism. Prooxidant-antioxidant balance (PAB) assay is a completely different test than oxidative stress or antioxidant capacity. In a specific pathologic or physiologic situation, oxidative stress or antioxidant markers may change, but it does not necessarily mean a change in the oxidant-antioxidant balance. PAB assay simultaneously measures total oxidants and antioxidants in one test, and this is the major difference between PAB and the traditional methods. So, the present study was designed to determine serum Se and PAB value and evaluate the association between serum Se with PAB value and hematological parameters in CH patients.

Material and Methods

Study population

We enrolled 39 patients aged three months to one year (20 females and 19 males) with CH and 60 non-CH children as the control group in this case-control study. Diagnosis of disease was initially identified by newborn screening and followed up in the pediatric clinic of Besat hospital, Sanandaj, Iran. In a routine neonatal screening for thyroid disorders, newborn screening was done with filter paper for CH between 72 h and 7 days past birth; blood samples were collected from the heel and thyroid-stimulating hormone (TSH) level was measured; positive results were confirmed by measurement of serum TSH and free T4 using an enzyme-linked immunosorbent assay (ELISA). For keeping serum TSH concentrations near to the normal range, a daily treatment with levothyrox-

ine was used for all patients (10–15 µg/kg/day). All parents of the studied neonates were informed about the survey and freely signed the consent form. The protocol of this study was agreed by the ethics committee of the Kurdistan University of Medical Sciences. The study was carried out according to the Declaration of Helsinki for Medical Research Involving Human Patients and approved by the local research ethics committee. Subjects with a history of congenital adrenal hyperplasia, infectious diseases, hypertension, signs or symptoms of other endocrinopathies were excluded from the study.

Blood sampling

The blood samples were collected, and serum was separated and stored at -70°C pending assay. Whole blood was used for whole blood cell count by a cell counter (Sysmex hematology analyzer kx-21, Sysmex Canada, Inc.). Serum levels of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) were measured with a specific enzyme-linked immunosorbent (ELISA) assay from Monobind Inc. (Lake Forest, CA 92630, United States).

Determination of serum Se concentration: Se concentration was determined in serum samples by atomic absorption spectroscopy.

Measurement of serum PAB value

PAB was determined according to a previously described method (12). Briefly, standard reagents were prepared by varying proportions (0–100%) of 250 µmol/L hydrogen peroxide with 3 mmol/L uric acid (in 10 mmol/L NaOH) and then standard curve was plotted using these reagents. Based on the hydrogen peroxide concentration in the reaction, the peroxidase enzyme oxidase 3,3,5,5-tetramethylbenzidine (TMB) substrate produced a visible blue dye. Finally, the reaction was stopped using an HCL reagent and the absorbance of yellowish product was read at 450 nm. The values of the PAB are shown as an arbitrary HK unit that was the percentage of H_2O_2 in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

Statistical analyses

All the data were statistically evaluated with SPSS (ver. 16). The Shapiro-Wilk test was applied to determine the assumption of normal distribution. Results were presented as Mean \pm SD if the normality assumption was met; otherwise, Median \pm IQR (intermediate quartile range) was used. The independent samples T-test or Mann-Whitney statistical tests were used to compare mean/median differences between two experimental groups. The relationship between each

pair of parameters was estimated using Spearman's rho correlation test. The results were analyzed by the factorial analysis of variance (ANOVA) test, where a p value ≤ 0.05 was considered statistically significant. If the normality assumption was violated, Kruskal-Wallis test was used instead.

Results

Thirty-nine CH patients and 60 healthy children were enrolled in the study as case and control groups. The patients in the present study were children with congenital hypothyroidism; 20 girls (51.3%) and 19 boys (48.7%), with a sex ratio of 1.05, and healthy subjects were 31 females (51.7%) and 29 males (48.3%) with a sex ratio of 1.06. Mean age of CH patients was 5.08 ± 1.58 months (range 3–12 months) and for controls 6.25 ± 1.48 months (range 1–15 months). There was no significant difference between the studied groups regarding sex and age (p value < 0.05).

Laboratory findings of patients and controls were presented in *Figure 1a-d*. Serum TSH concentrations in patient and control groups were 4.35 (3.425) and 1.8 (2.2) U/mL, respectively (*Figure 1a*). Besides, FT4 was 0.09 (0.045) ng/L in CH patients and 0.1 (0.05) ng/L in the healthy group (*Figure 1b*). Serum TSH but not FT4 levels had statistically significant differences between the studied subjects (p value = 0.002 and p value = 0.067, respectively). TSH concentration in male and female subjects was 2.5 (3.975) U/mL and 2.4 (3.3) U/mL. Furthermore, FT4 levels in boys and girls were 0.08 (0.05) ng/L and 0.08 (0.05) ng/L respectively. There were no statistically significant differences between males and females for TSH and FT4 (p value = 0.454 and 0.288, respectively).

We also measured the serum PAB value in both patients and healthy controls. According to our data, PAB levels were higher (140.00 (54.00) HK) in patients compared to controls (64.3 (161.52) HK) (p value = 0.001) (*Figure 1c*). In addition, measuring of serum Se in patients and controls showed that patients had lower (10.72 (4.058) µg/L) Se levels compared to controls (22.14 (82.656) µg/L) (p value < 0.011) (*Figure 1d*). There were no statistically significant differences for serum PAB value in boys (117.00 (162.1) HK) and girls (117.00 (153.6) HK) (p value = 0.671). In addition, serum Se concentration in male (12.912 (38.45) µg/L) and female (12.912 (55.05) µg/L) subjects had no significant difference (p value = 0.287).

At the next step of our study, we assessed median (IQR) values of hematological indices including WBC and RBC counts, hematocrit (Hct), Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (Plt), neutrophils (Neut) and lym-

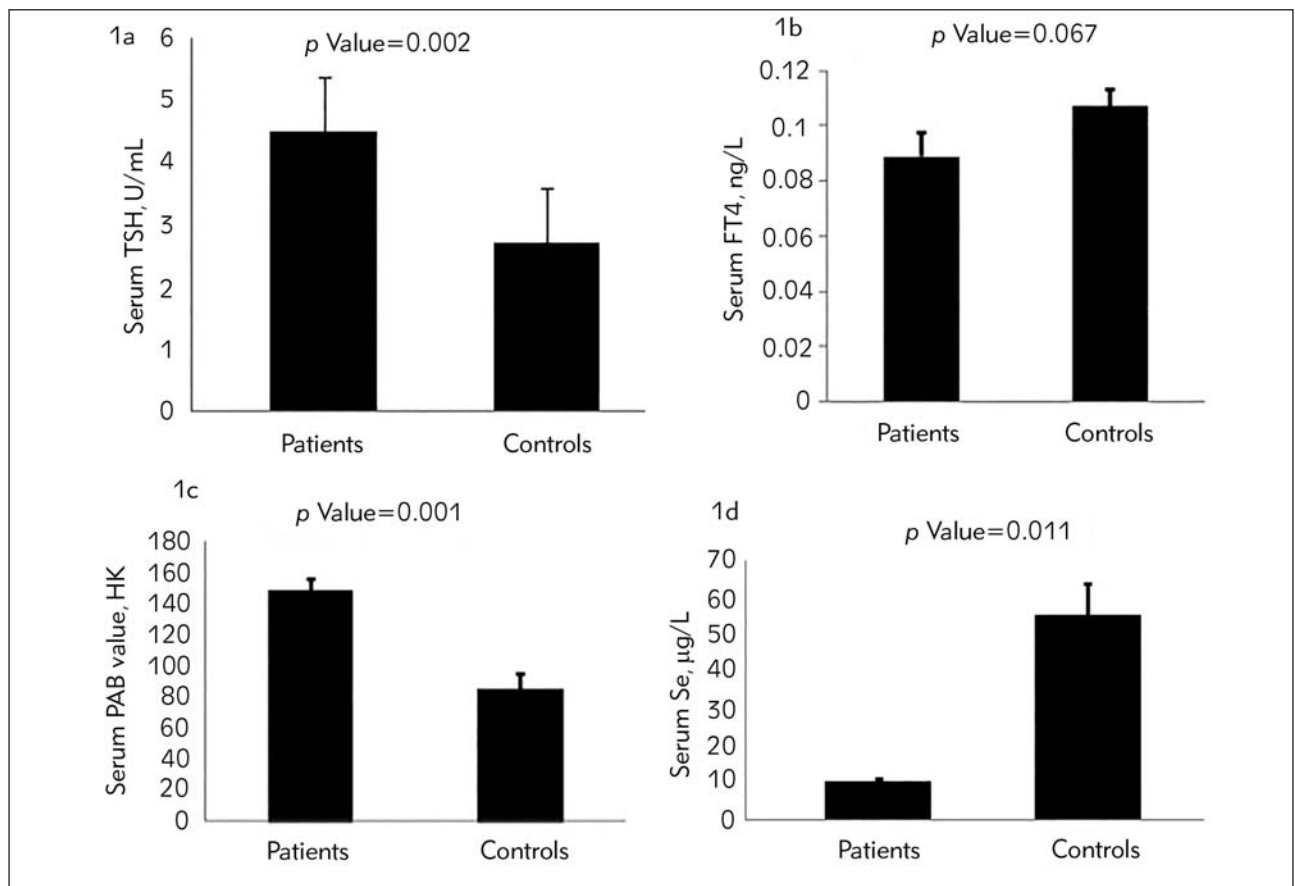


Figure 1 Biochemical findings in studied groups (Mean \pm SEM): 1a: Serum TSH levels (in U/mL unit) are indicated as a block value that was in a higher concentration (4.48 ± 0.69 U/mL) for the patient group and decreasing concentration to 2.7 ± 0.45 U/mL for controls with a significant p value=0.002 for their difference. 1b: According to the plot, serum FT4 has no significant differences (p value=0.067) between patients (0.089 ± 0.0083 ng/L) and controls (0.107 ± 0.0062 ng/L). 1c: The difference between serum PAB value (in HK unit) in patients (148.35 ± 7.05 HK) and controls (84.51 ± 10.51 HK) was also significant (p value=0.001). 1d: Serum Se concentration in patient subjects was lower (10.5 ± 0.65 μ g/L) than in controls (55.17 ± 8.55 μ g/L) and this difference was also significant (p value = 0.011).

Table I Hematological indices in case and control groups.

	CH patients (n=39)	Healthy controls (n=60)	p value
WBC count ($\times 10^3$ cell/ μ L)	6.5 (2.3)	8.3 (5.457)	0.032
RBC count ($\times 10^6$ cell/ μ L)	4.675 (0.648)	4.68 (0.4)	0.659
Hb (g/L)	1.121 (0.1675)	1.23 (0.145)	0.079
Hct (%)	35.00 (4.775)	37.4 (4.1)	0.479
MCV (fL/cell)	75.82 (5.765)	79.9 (4.9)	0.066
MCH (pg/cell)	24.71 (2.335)	26.1 (1.9)	0.064
MCHC (g/L)	3.27 (0.166)	3.25 (0.23)	0.821
Plt count ($\times 10^5$ / μ L)	2.71 (0.72)	3.34 (8.153)	<0.0001
Lym ($\times 10^3$ cell/ μ L)	3.00 (1.9)	5.7 (1.7)	<0.0001
Neut ($\times 10^3$ cell/ μ L)	3.15 (1.95)	3.00 (4.5)	0.076
RDW-CV (%)	12.8 (0.7)	13.3 (1.675)	0.087

WBC: White blood cell; RBC: red blood cell; Plt: platelets; RDW: red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Hb: hemoglobin; Hct: hematocrit; Lym: lymphocyte; Neut: neutrophil; IQR: intermediate quartile range. Data is presented as median (IQR).

Table II Correlation between PAB and Se with biochemical and hematological markers in studied subjects.

	PAB (HK)	Se ($\mu\text{g/L}$)	TSH (U/mL)	FT4 (ng/L)
TSH (U/mL)	Spearman's rho = 0.006 p value = 0.958	Spearman's rho = 0.125 p value = 0.289	-	-
FT4 (ng/L)	Spearman's rho = 0.025 p value = 0.814	Spearman's rho = 0.072 p value = 0.542	-	-
WBC count ($\times 10^3$ cell/mL)	Spearman's rho = -0.177 p value = 0.098	Spearman's rho = 0.142 p value = 0.223	Spearman's rho = -0.092 p value = 0.397	Spearman's rho = 0.08 p value = 0.459
RBC count ($\times 10^6$ cell/mL)	Spearman's rho = 0.170 p value = 0.110	Spearman's rho = 0.058 p value = 0.622	Spearman's rho = 0.034 p value = 0.755	Spearman's rho = -0.145 p value = 0.176
Hb (g/L)	Spearman's rho = -0.065 p value = 0.543	Spearman's rho = 0.081 p value = 0.487	Spearman's rho = 0.171 p value = 0.113	Spearman's rho = -0.071 p value = 0.506
Hct (%)	Spearman's rho = -0.057 p value = 0.598	Spearman's rho = 0.101 p value = 0.39	Spearman's rho = 0.190 p value = 0.077	Spearman's rho = 0.000 p value = 0.998
MCV (fL/cell)	Spearman's rho = -0.066 p value = 0.539	Spearman's rho = 0.269 p value = 0.019	Spearman's rho = 0.167 p value = 0.123	Spearman's rho = 0.104 p value = 0.331
MCH (pg/cell)	Spearman's rho = -0.105 p value = 0.33	Spearman's rho = 0.160 p value = 0.171	Spearman's rho = 0.74 p value = 0.494	Spearman's rho = -0.023 p value = 0.830
MCHC (g/L)	Spearman's rho = -0.144 p value = 0.179	Spearman's rho = 0.053 p value = 0.652	Spearman's rho = 0.061 p value = 0.574	Spearman's rho = -0.150 p value = 0.159
Plt count ($\times 10^5$ /mL)	Spearman's rho = -0.06 p value = 0.576	Spearman's rho = 0.051 p value = 0.665	Spearman's rho = 0.307 p value = 0.004	Spearman's rho = 0.341 p value = 0.001
Lym ($\times 10^3$ cell/mL)	Spearman's rho = -0.092 p value = 0.442	Spearman's rho = 0.052 p value = 0.689	Spearman's rho = 0.413 p value < 0.0001	Spearman's rho = 0.249 p value = 0.035
Neut ($\times 10^3$ cell/mL)	Spearman's rho = -0.032 p value = 0.810	Spearman's rho = 0.057 p value = 0.685	Spearman's rho = 0.104 p value = 0.432	Spearman's rho = 0.199 p value = 0.125
RDW-CV (%)	Spearman's rho = -0.217 p value = 0.152	Spearman's rho = 0.136 p value = 0.414	Spearman's rho = -0.034 p value = 0.828	Spearman's rho = 0.392 p value = 0.008

TSH: thyroid-stimulating hormone; FT4: free thyroxine; Se: selenium; PAB: prooxidant-antioxidant balance; WBC: white blood cell; RBC: red blood cell; Plt: platelets; RDW: red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Hb: hemoglobin; Hct: hematocrit; Lym: lymphocyte; Neut: neutrophil.

phocytes (Lym) in both studied groups. Results are shown in *Table I*. Comparison between control and patient groups revealed that there was a statistically significant difference in WBC and Plt counts and Lym parameter. RBC count, Hct, and Hb parameters did not significantly differ in CH patients and healthy controls, although statistical analysis showed a trend to lower levels of these indices in CH patients (*Table II*).

Table II shows the correlation between PAB values with serum TSH concentration. No statistically significant correlation was observed between PAB values and TSH levels (rho = 0.006, p value = 0.958). Besides, there was no statistically significant correla-

tion between Se concentration with TSH level (rho = 0.125, p value = 0.289) and FT4 concentration (rho = 0.072, p value = 0.542). There was a significant correlation between PAB value and serum Se concentration (*Figure 2*), and also between Se concentration and MCV, however, no significant correlation was found between PAB levels and hematological indices (for all parameters, p value > 0.05) (*Table II*). We also assessed the possible correlation between serum TSH and FT4 with hematological findings in the studied subjects. Our results showed that there was a positive correlation between serum TSH with Plt and Lym and also a significant correlation between FT4 with Plt count, Lym and RDW (*Table II*).

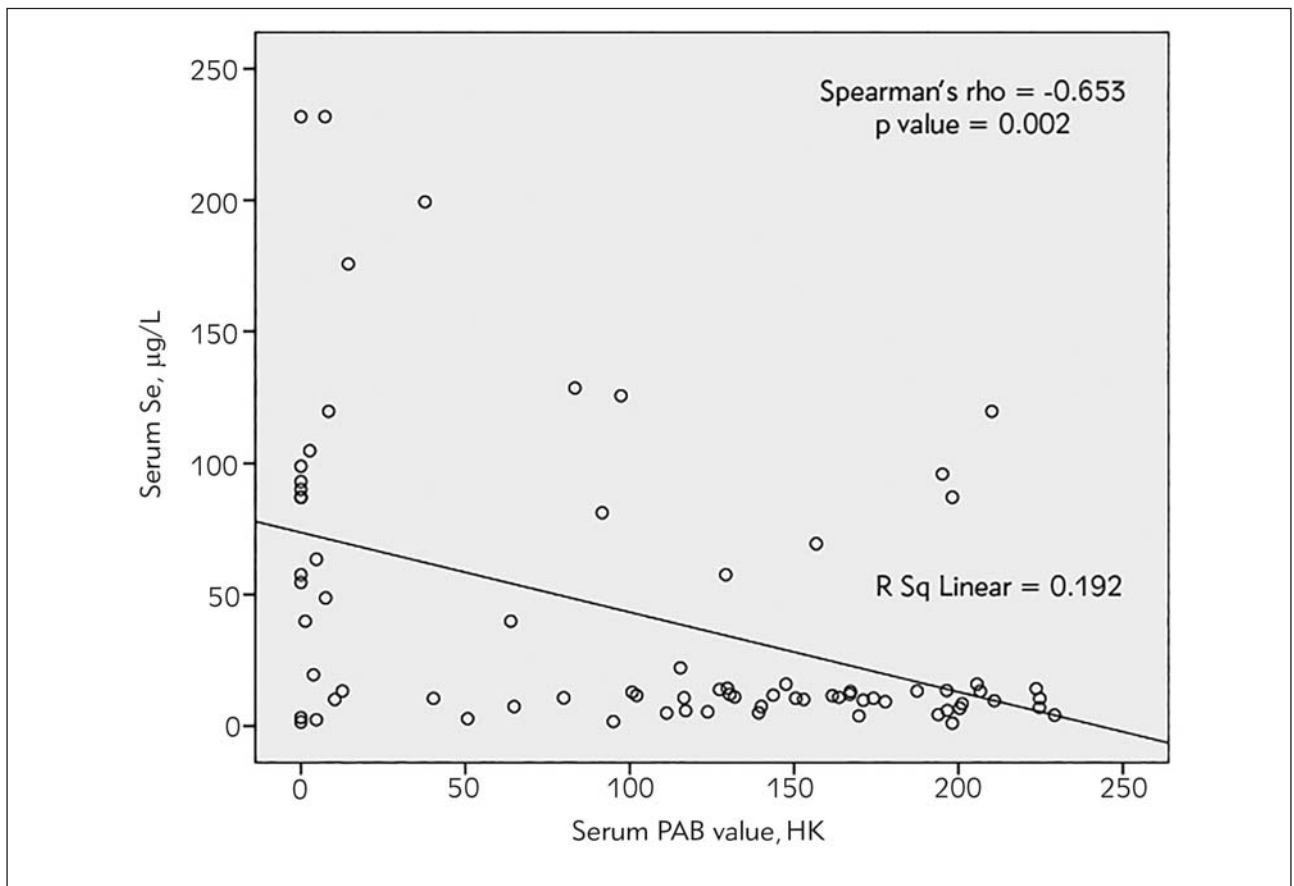


Figure 2 Association between serum Se concentrations with serum PAB value: According to regression analysis, Se level (in $\mu\text{g}/\text{mL}$ unit) had an indirect correlation with serum PAB value (in HK unit). The Spearman correlation showed that Se levels correlated significantly with PAB levels ($r = -0.653$, p value = 0.002).

Discussion

In the present study, we evaluated the oxidant-antioxidant status in patients with congenital hypothyroidism by measuring serum PAB and Se levels and saw that these patients had decreased Se levels, along with increased PAB values. In addition, there was a significant difference in some hematological indices between groups. Total status of oxidants and antioxidants in a specific biological fluid can be measured by a single PAB assay. To the best of our knowledge, our study is the first to investigate the PAB value, Se concentration, hematological indices and their association in patients with congenital hypothyroidism.

Previous studies revealed that changes in thyroid hormones increase the production of prooxidants (14–16). Besides, it has been shown that higher prooxidants levels such as ROS and free radicals result in many pathological outcomes (17, 18). Selenium plays an important role as an antioxidative agent in decreasing the elevated prooxidant levels resulting from thyroid gland dysfunction (19). Both hyper- and hypothyroidism may lead to increased oxidative stress.

Higher production of ROS and decreased level of antioxidants such as SOD and glutathione are the main reasons for increased oxidative stress in hyperthyroid and hypothyroid patients, respectively (20, 21).

In hyperthyroidism, it was shown that oxidative stress markers are correlated with TSH and thyroid hormone concentrations (22). Furthermore, it seems that ROS-induced oxidative stress is highly related to Graves ophthalmopathy (23), so even after treatment and correction of thyroid hormones increased levels of oxidative damage may remain (24).

On the other hand, with regard to hypothyroidism, results are still inconsistent. Although some studies showed that because of the lower metabolic rate in hypothyroidism, the free radical generation and hence oxidative stress decrease (25, 26), others stated that decreased levels of antioxidants in patients with hypothyroidism cause an increased oxidative stress (20, 27, 28). Baser et al. previously showed that compared to healthy controls in the serum of euthyroid Hashimoto patients oxidant status was significantly increased, and positively correlated with the levels of thyroglobulin antibodies (29).

Due to additive effects of prooxidants and antioxidants on each other, it is essential to simultaneously measure total oxidants and antioxidants in a single test, and this is the priority of the PAB assay in relation to other oxidative stress markers. In a specific situation, the value of each oxidative stress marker may change alone, but the total balance of oxidants and antioxidants remains constant. Higher PAB value in a specific biological fluid shows that the level of oxidants is increased more than antioxidants. Increased levels of PAB have been reported in different pathologic and physiologic situations such as phenylketonuria, polycystic ovary syndrome and coronary artery disease (11, 12, 30). There are no other studies evaluating PAB levels in thyroid disorders and to the best of our knowledge, the present study is the first study which assessed the PAB value in CH patients. In our study and in line with previous data, PAB levels were found to be higher in patients with CH compared to healthy subjects, however, no statistically significant association was found between serum PAB values and serum TSH or FT4 concentration. A possible cause for this situation could be explained by the sample size or the type of samples. Although sampling was conducted from the entire CH population, a larger CH population could be useful. In addition, for the measurement of Se concentration, sampling from the tissue is better.

Selenium is an essential trace mineral and as a co-enzyme of many selenoproteins is involved in antioxidant defense mechanisms, thyroid metabolism and the immune function. Previous studies have revealed that selenium supplementation may be used as a modification tool for the management of autoimmune thyroid disorders (31, 32). Although the decreased level of selenium in thyroid tissue has been shown in different thyroid disorders, there is a paucity of studies with regard to the impact of Se on the pathogenesis of CH. It seems that tissue conversion of T4 to T3 is not affected by the decreased level of selenium in CH patients (33), however, selenium supplementation may improve thyroid hormones feedback at the pituitary level and decrease stimulation of the residual thyroid tissue probably indicating the higher cellular conversion of T4 to T3 in CH patients (34). In the present study, we showed that serum Se concentration dramatically decreased in CH patients compared to healthy controls. Furthermore, our results showed that there was a statistically significant association between Se concentration and PAB values.

Anemia is a frequent outcome in adult patients with hypothyroidism. Some studies showed that anemia is a common finding in patients with CH (35, 36). Franzese et al. (35) showed that hematologic parameters including Hct, Hb and RBC count were in the lower range of the reference interval. After treatment with L-thyroxine, CH patients with T4 concentration at first diagnosis lower than 0.3 µg/L had lower Hct, Hb and RBC count than patients with T4 ≥

0.3 µg/L at diagnosis. These parameters were raised at 6 and 12 months after treatment. They proved that the hematological indices had a significant correlation with thyroid hormones levels at diagnosis and these values might affect the hematologic parameters even after treatment has begun. Accordingly, our results showed that there was a trend to a decreased RBC count, Hct, Hb in patients compared to controls. Furthermore, normal MCV in CH patients suggested a normocytic anemia. Our findings are in contrast to Onyiriuka (36) study which revealed that CH patients may develop severe anemia and this possibility should be considered for all anemic infancies with uncertain etiology. Impaired absorption of iron, vitamin B12, and folic acid are the possible reasons for persistent anemia even after treatment with levothyroxine. Moreover, based on our results, change in PAB value may not be considered as a potential risk factor for anemia in CH patients, however, this finding needs further studies.

In conclusion, the prooxidant-antioxidant balance is in favor of the oxidative side in patients with congenital hypothyroidism. In addition, our results suggest that lower selenium concentration is likely to be associated with increased PAB values. It seems that treatment of CH patients can effectively compensate hormone imbalance and hematologic disorders; however, alterations of Se and PAB are still present and may cause pathological disorders in these patients. Our study had some limitations. First, our study population was rather small and second, we did not measure the Se level in thyroid tissue. So, further studies with higher statistical power and measurement of Se in the tissue samples of patients with congenital hypothyroidism are required.

Acknowledgments: The author wishes to thank all the patients and health staff who participated in this study. Financial support from the Ministry of Health and Medical Education (MOHME) of Iran is highly appreciated.

Disclosure of potential conflicts of interest

Mrs. Sh Rostami declares that he has no conflict of interest. Dr. A Fathollahpour declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Dr. M Abdi has received research grants from the Ministry of Health and Medical Education (MOHME) of Iran. Mrs. K Naderi declares that she has no conflict of interest.

Ethical approval

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the ethics committee of Kurdistan University of Medical Sciences and with the 1964

Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Funding source

This work was supported by a research grant from the Ministry of Health and Medical Education (MOHME) of Iran (Grant/Award Number: '1395/305').

Financial disclosure

The author has no financial relationships relevant to this article to disclose.

Author Contributions

All authors contributed equally to this work.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

- Rastogi MV, LaFranchi SH. Congenital hypothyroidism. *Orphanet J Rare Dis* 2010; 5: 17.
- Mirjanic-Azaric B, Avram S, Stojakovic-Jelisavac T, Stojanovic D, Petkovic M, Bogavac-Stanojevic N, Ignjatovic S, Stojanov M. Direct estimation of reference intervals for thyroid parameters in the Republic of Srpska. *J Med Biochem* 2017; 36: 137–44.
- Hulur I, Hermanns P, Nestoris C, Heger S, Refetoff S, Pohlenz J, et al. A single copy of the recently identified dual oxidase maturation factor (duoxa) 1 gene produces only mild transient hypothyroidism in a patient with a novel biallelic duoxa2 mutation and monoallelic duoxa1 deletion. *J Clin Endocrinol Metab* 2011; 96: E841–845.
- Jacob H, Peters C. Screening, diagnosis and management of congenital hypothyroidism: European society for paediatric endocrinology consensus guideline. *Arch Dis Child Educ Pract Ed* 2015; 100: 260–3.
- Ghasemi M, Hashemipour M, Hovsepian S, Heiydari K, Sajadi A, Hadian R, et al. Prevalence of transient congenital hypothyroidism in central part of Iran. *J Res Med Sci* 2013; 18: 699–703.
- Cattani D, Goulart PB, Cavalli VL, Winkelmann-Duarte E, Dos Santos AQ, Pierozan P, et al. Congenital hypothyroidism alters the oxidative status, enzyme activities and morphological parameters in the hippocampus of developing rats. *Mol Cell Endocrinol* 2013; 375: 14–26.
- Cacciatore I, Baldassarre L, Fornasari E, Mollica A, Pinnen F. Recent advances in the treatment of neurodegenerative diseases based on gsh delivery systems. *Oxid Med Cell Longev* 2012; 2012: 240146.
- Bashir H, Bhat MH, Farooq R, Majid S, Shoib S, Hamid R, et al. Comparison of hematological parameters in untreated and treated subclinical hypothyroidism and primary hypothyroidism patients. *Med J Islam Repub Iran* 2012; 26: 172–8.
- Dorgalaleh A, Mahmoodi M, Varmaghani B, Kiani Node F, Saeedi Kia O, Alizadeh S, et al. Effect of thyroid dysfunctions on blood cell count and red blood cell indices. *Iran J Ped Hematol Oncol* 2013; 3: 73–7.
- Iddah MA, Macharia BN, Ng'wena AG, Keter A, Ofulla AV. Thyroid hormones and hematological indices levels in thyroid disorders patients at Moi Teaching and Referral Hospital, Western Kenya. *ISRN Endocrinol* 2013; 2013: 385940.
- Heshmati N, Shahgheibi S, Nikkhoo B, Amini S, Abdi M. Association of prooxidant-antioxidant balance with clinical and laboratory parameters and its relation to different drug regimens in polycystic ovary syndrome women with normal BMI. *Indian J Clin Biochem* 2016: 1–8.
- Tavana S, Amini S, Hakhamaneshi MS, Andalibi P, Hajir MS, Ardalan A, et al. Prooxidant-antioxidant balance in patients with phenylketonuria and its correlation to biochemical and hematological parameters. *J Pediatr Endocrinol Metab* 2016; 29: 675–80.
- Naji P, Kumar G, Dewani S, Diedrich WA, Gupta A. Graves' disease causing pancytopenia and autoimmune hemolytic anemia at different time intervals: A case report and a review of the literature. *Case Rep Med* 2013; 2013: 194542.
- Pandrc MS, Ristić A, Kostovski V, Stanković M, Antić V, Milin-Lazović J, Ćirić J. The Effect of Early Substitution of Subclinical Hypothyroidism on Biochemical Blood Parameters and the Quality of Life. *J Med Biochem* 2017; 36: 127–36.
- Santi A, Duarte MM, de Menezes CC, Loro VL. Association of lipids with oxidative stress biomarkers in subclinical hypothyroidism. *Int J Endocrinol* 2012; 2012: 856359.
- Mancini A, Di Segni C, Raimondo S, Olivieri G, Silvestrini A, Meucci E, et al. Thyroid hormones, oxidative stress, and inflammation. *Mediators Inflamm* 2016; 2016: 6757154.
- Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. *ISRN Oncol* 2012; 2012: 137289.
- Parascandolo A, Rappa F, Cappello F, Kim J, Cantu DA, Chen H, et al. Extracellular superoxide dismutase expression in papillary thyroid cancer mesenchymal stem/stromal cells modulates cancer cell growth and migration. *Sci Rep* 2017; 7: 41416.
- Ventura M, Melo M, Carrilho F. Selenium and thyroid disease: From pathophysiology to treatment. *Int J Endocrinol* 2017; 2017: 1297658.

20. Reddy VS, Gouroju S, Suchitra MM, Suresh V, Sachan A, Srinivasa Rao PV, et al. Antioxidant defense in overt and subclinical hypothyroidism. *Horm Metab Res* 2013; 45: 754–8.
21. Saad-Hussein A, Hamdy H, Aziz HM, Mahdy-Abdallah H. Thyroid functions in paints production workers and the mechanism of oxidative-antioxidants status. *Toxicol Ind Health* 2011; 27: 257–63.
22. Aslan M, Cosar N, Celik H, Aksoy N, Dulger AC, Begenik H, et al. Evaluation of oxidative status in patients with hyperthyroidism. *Endocrine* 2011; 40: 285–9.
23. Marcocci C, Bartalena L. Role of oxidative stress and selenium in Graves' hyperthyroidism and orbitopathy. *J Endocrinol Invest* 2013; 36: 15–20.
24. Kaur A, Pandey S, Kumar S, Mehdi AA, Mishra A. Oxidative stress profile in Graves' ophthalmopathy in Indian patients. *Orbit* 2010; 29: 97–101.
25. Sarati LI, Toblli JE, Martinez CR, Uceda A, Feldman M, Balaszczuk AM, et al. Nitric oxide and aqp2 in hypothyroid rats: A link between aging and water homeostasis. *Metabolism* 2013; 62: 1287–95.
26. Kong L, Wei Q, Fedail JS, Shi F, Nagaoka K, Watanabe G. Effects of thyroid hormones on the antioxidative status in the uterus of young adult rats. *J Reprod Dev* 2015; 61: 219–27.
27. Novakovic TR, Dolicanin ZC, Djordjevic NZ. Oxidative stress biomarkers in amniotic fluid of pregnant women with hypothyroidism. *J Matern Fetal Neonatal Med* 2017: 1–6.
28. Villanueva I, Alva-Sanchez C, Pacheco-Rosado J. The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. *Oxid Med Cell Longev* 2013; 2013: 218145.
29. Baser H, Can U, Baser S, Yerlikaya FH, Aslan U, Hidayetoglu BT. Assessment of oxidative status and its association with thyroid autoantibodies in patients with euthyroid autoimmune thyroiditis. *Endocrine* 2015; 48: 916–23.
30. Nabatchican F, Einollahi N, Kazemi Khaledi A. Relationship between prooxidant-antioxidant balance and severity of coronary artery disease in patients of Imam Khomeini Hospital of Tehran, Iran. *Acta Med Iran* 2014; 52: 116–21.
31. Wang W, Xue H, Li Y, Hou X, Fan C, Wang H, et al. Effects of selenium supplementation on spontaneous autoimmune thyroiditis in nod.H-2h4 mice. *Thyroid* 2015; 25: 1137–44.
32. Drutel A, Archambeaud F, Caron P. Selenium and the thyroid gland: More good news for clinicians. *Clin Endocrinol (Oxf)* 2013; 78: 155–64.
33. Chanoine JP, Neve J, Wu S, Vanderpas J, Bourdoux P. Selenium decreases thyroglobulin concentrations but does not affect the increased thyroxine-to-triiodothyronine ratio in children with congenital hypothyroidism. *J Clin Endocrinol Metab* 2001; 86: 1160–3.
34. Chanoine JP. Selenium and thyroid function in infants, children and adolescents. *Biofactors* 2003; 19: 137–3.
35. Franzese A, Salerno M, Argenziano A, Buongiovanni C, Limauro R, Tenore A. Anemia in infants with congenital hypothyroidism diagnosed by neonatal screening. *J Endocrinol Invest* 1996; 19: 613–9.
36. Onyiriuka AN, Kouyate M, Oduwole AO, Oyenusi EE, Oluwayemi IO, Fakaye-Udeogu OB, et al. Primary congenital hypothyroidism complicated by persistent severe anaemia in early infancy: A case report with a literature review. *Journal of Endocrinology, Metabolism and Diabetes of South Africa* 2014; 19: 85–8.

Received: October 4, 2017

Accepted: November 20, 2017