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# Association of maternal serum trace elements with newborn screening-thyroid stimulating hormone

## [Yenidoğan Tiroid Stimulan Hormon Tarama Sonuçları ile Anne Serum Eser Element Düzeylerinin İlişkisi]

<https://doi.org/10.1515/tjb-2019-0372>

Received September 11, 2019; accepted November 28, 2019;

published online June 23, 2020

### Abstract

**Objectives:** Trace elements are essential in thyroid functioning as they incorporate into biologically important enzymes as cofactors. The placenta can either activate or inhibit the transfer of maternal trace elements to the unborn. An imbalance of maternal trace elements in pregnancy may affect both maternal and newborn thyroid function.

**Methods:** Blood samples from 315 lactating mothers were collected in the first 48 h after delivery and evaluated for selenium (Se), copper (Cu), manganese (Mn), and zinc (Zn) using flame atomic absorption spectroscopy (FAAS) and quadrupole inductively coupled plasma-mass spectrometer (ICP-MS). Thyroid hormones and auto-antibodies

(thyroid-stimulating hormone (TSH), free T<sub>3</sub> (fT<sub>3</sub>), free T<sub>4</sub> (fT<sub>4</sub>), anti-thyroid peroxidase (anti-TPO), and antithyroglobulin (anti-TG)) were analyzed in maternal blood using an electro-chemiluminescence immunoassay (ECLIA). Between 48 and 72 postpartum hours, spot blood samples were used for newborn screening-TSH measurement. Correlation and multivariate analyses were performed to evaluate the effect of maternal trace element levels on newborn screening-TSH levels.

**Results:** The medians (min-max) of maternal Se (45.16 µg/L (21.28–79.04)), Cu (210.10 µg/dL (117.04–390.64)), Mn (2.11 µg/L (0.20–3.46)), and Zn (0.43 mg/L (0.24–0.66)) were determined. A positive correlation was detected between Zn and maternal TSH levels ( $r=0.12$ ,  $p < 0.05$ ). Newborn screening-TSH was significantly correlated with maternal Cu ( $r=0.14$ ,  $p < 0.01$ ). Similarly, Cu exhibited weak associations in clustering analysis while others shared common clusters with newborn-screening TSH.

**Conclusions:** There was no significant association between most of the maternal serum trace elements and maternal thyroid hormone parameters, with an only exception between maternal Zn and maternal serum TSH. Finally, the association between maternal serum Cu levels and newborn screening-TSH levels may highlight the importance of maternal Cu levels on the newborn thyroid health.

**Keywords:** copper levels; maternal thyroid hormones; newborn screening-TSH; trace elements.

### Öz

**Amaç:** Eser elementler, biyolojik öneme sahip enzimlerin yapısına kofaktör olarak dahil olur ve bu elementlerin tiroid fonksiyonunda da önemli görevleri vardır. Plasenta,

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anne eser elementlerinin fetüse transferini sağlar veya engeller. Gebelik süresinde anneye ait eser elementlerin dengesizliği, hem anne hem de yenidoğanın tiroid fonksiyonunu etkileyebilir.

**Gereç ve Yöntem:** Doğumdan sonraki ilk 48 saatte, 315 anneden toplanan kan örnekleri, alev atomik absorpsiyon spektroskopisi (FAAS) ve indüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) kullanılarak selenyum (Se), bakır (Cu), manganez (Mn) ve çinko (Zn) açısından değerlendirildi. Tiroid hormonları ve otoantikörleri (TSH, fT3, fT4, anti-TPO ve anti-TG) elektrokemilüminesans immünoanaliz yöntemiyle tayin edildi. Yenidoğan TSH tarama sonuçları kullanılarak, anne serum eser element düzeylerinin yenidoğan TSH düzeylerine etkisi korelasyon ve çok değişkenli analizler ile belirlendi.

**Bulgular:** Anne serum Se (45.16 µg/L (21.28–79.04)), Cu (210.10 µg/dL (117.04–390.64)), Mn (2.11 µg/L (0.20–3.46)) ve Zn (0.43 mg/L(0.24–0.66)) medyan değerleri (min-max) tespit edildi. Anne TSH düzeyi ile Zn düzeyi arasında pozitif korelasyon saptandı ( $r=0.12$ ,  $p < 0.05$ ). Yenidoğan TSH taraması sonucu, anne Cu düzeyi ile anlamlı korelasyon gösterdi ( $r=0.14$ ,  $p < 0.01$ ). Benzer şekilde, kümeleme analizine göre Cu diğer iz elementlere kıyasla yenidoğan TSH ile daha zayıf kümelendi.

**Sonuç:** Anne serum eser elementlerinin çoğu ile anne tiroid hormonu parametreleri arasında anlamlı bir ilişki bulunmamakla birlikte, anne Zn ve anne serum TSH seviyesi arasında istatistiksel anlamlı bir ilişki bulunmaktadır. Son olarak, anne serum Cu düzeyleri ile yenidoğan TSH tarama sonucu arasındaki ilişki, anne Cu düzeyinin yenidoğan tiroid sağlığı üzerindeki önemini vurgulamaktadır.

**Anahtar Kelimeler:** eser element; yenidoğan TSH tarama testi; maternal tiroid hormonu; bakır seviyesi.

## Introduction

The thyroid hormones thyroxine (T4) and triiodothyronine (T3) are essential for the normal development and growth of the human newborn. Abnormalities of thyroid gland function result in metabolic consequences of thyroid dysfunction and mental deficiencies in infants [1]. Thyroid hormone secretion is regulated by the thyroid-stimulating hormone (TSH) and disorders at either TSH or thyroid hormone secretion can lead to thyroid gland diseases. Trace elements are important in health and disease state as most of them incorporate into biologically important enzymes as cofactors [2–4]. For example, during iodine organification in thyroid hormone synthesis, hydrogen peroxide, one of the major reactive oxygen species (ROS) is

excessively generated in the thyroid gland [5]. The damaging effect of ROS is eliminated mainly by the antioxidant enzymatic system, which relies on trace elements such as selenium (Se), copper (Cu), manganese (Mn), and zinc (Zn) as cofactors [6]. Hence, any change in trace element balance can disrupt thyroid hormone functioning and results in various thyroid disorders in adults and infants [7]. It is well established that the barrier function of the placenta either activates or inhibits the transfer of maternal trace elements to the unborn [6]. Therefore, an imbalance of maternal trace elements may affect both maternal and newborn thyroid function and health.

Selenium is an essential trace element that incorporates into deiodinases and contributes to thyroid hormone homeostasis. The content of Se in tissues such as thyroid, adrenals, testes, ovary, and pituitary is high; the thyroid gland having the highest content of Se [3]. Copper, another important element required for normal growth and development, is a component of several important biochemical enzymes and actively participates in the antioxidant system [8]. For example, Cu/Zn Dismutase can effectively eliminate ROS and support the antioxidant balance in the human body [8]. Similarly, Mn is a cofactor of many important enzymes, especially Mn-Superoxide Dismutase, a major enzyme in detoxifying ROS [9].

Given the importance of trace elements in thyroid homeostasis and limited literature information regarding trace element levels in the sensitive population such as lactating women and their newborns, we evaluated the effect of maternal trace element levels (Se, Cu, Mn, and Zn) on newborn thyroid health status.

## Materials and methods

### Recruitment of subjects and sample collection

Healthy pregnant women (n=315) with a singleton pregnancy in week 38 were recruited at Zeynep Kamil Research and Training Hospital, Istanbul-Turkey, a state hospital specialized in women obstetrics and gynecology between June 2013-August 2013. Any pregnant woman with vitamin supplementation, thyroid disorder, chronic illnesses, and under thyroid-active medication (e. g., amiodarone, glucocorticoids, dopamine, propranolol, iodine, lithium, phenytoin, and carbamazepine) was excluded from the study. All subjects gave informed consent and this study was approved by the Acibadem University Ethics Committee (ATADEK 2013-507).

### Sample collection and laboratory measurements

In the first 48 postpartum hours, maternal blood samples (3–5 mL) were obtained in blood serum tubes. The blood samples were

centrifuged for 15 min at 1800xg. Following centrifugation, the serum was separated into another tube. Newborn heel-prick blood was obtained between 48 and 72 h after delivery for newborn TSH measurement. Newborn screening-TSH was considered high ( $>5 \mu\text{U/mL}$ ) and normal ( $<5 \mu\text{U/mL}$ ) based on the cut-off levels [10, 11]. Newborn screening-TSH measurements were performed as part of the screening program using a fluorometric enzyme immunoassay kit (Trimaris Neonatal TSH FEIA, Bome, Turkey). All the samples were kept at  $-80^\circ\text{C}$  until analysis.

Maternal free T3 (fT3), free T4 (fT4), TSH, anti-thyroid peroxidase (TPO) antibodies, and anti-thyroglobulin (anti-Tg) antibodies from serum samples were determined by electro-chemiluminescence immunoassay (ECLIA) using Elecsys 2010 (Roche Diagnostics, Germany).

### Quantification of trace elements

All reagents and solvents were of ultrahigh purity grade and purchased from Merck (KGaA, Darmstadt, Germany) unless otherwise stated. For Zn and Cu measurements, serum samples were diluted five times with deionized water. Serum Zn and Cu levels were determined using a flame atomic absorption spectroscopy (FAAS) (Shimadzu AA-6200, Kyoto, Japan) equipped with a deuterium background correction and hollow cathode lamps as light sources. The instrument settings for serum Zn and Cu levels are given in Table 1. Argon was used as the purging gas. Serum Zn and Cu levels were calculated using calibration curves for each element from standard solutions [(Certificate Zinc Standard for AAS (Sigma Aldrich, Catalog No: 18827) and Copper Standard (1,000  $\mu\text{g/mL}$  in 2–5% Nitric acid, Accustandard, Inc USA, Catalog No: AA15N1)]. For both trace elements, the measurement modes were dependent on peak height.

For Mn and Se measurements, serum samples were diluted with 20-fold with triton buffer composed of 4% w/v n-butanol, 2% w/v ammonium hydroxide, 0.1% w/v Triton X-100 and 0.1% w/v EDTA. The trace elements were measured using a quadrupole inductively coupled plasma-mass spectrometer (ICP-MS) (Agilent 7700-Santa California, USA) equipped with a concentric glass nebulizer and a cyclonic spray chamber. The standards were used for the preparation of calibration standards (Se standard 10,000  $\mu\text{g/mL}$ , Mn standard 1,000  $\mu\text{g/mL}$  in 2–5% nitric acid from Accustandard, Inc USA, Catalog No: ICP-51N-1 and ICP-33N-1, respectively).

### Statistical analysis

Descriptive statistics including mean, median, 1st and 3rd quartiles, and standard deviation were used to evaluate subjects' demographic and clinical variables. Shapiro–Wilk test was applied for assessing data normality. The relationship between subjects' clinical variables and trace elements was performed using Pearson and Spearman

correlation analysis (Analyze-it Software, Ltd. United Kingdom, V4.20.1). Significance was set at  $p \leq 0.05$ .

For multivariate analysis, clustering analysis using single linkage and multiple regressions were performed. Bonferroni correction was applied to reduce type I errors in the multiple tests, considering  $p \leq 0.05$  as significantly different (SPSS PASW Statistics v18, IBM).

## Results

### Subject demographics and maternal and newborn thyroid test results

The subject demographics including maternal age, body mass index (BMI), newborn birth weight, maternal thyroid function test results (fT3, fT4, TSH, anti-TPO, and anti-Tg), and newborn screening-TSH levels are summarized in Table 2.

### Association between maternal thyroid function test results and maternal trace elements

The median Se ( $\mu\text{g/L}$ ), Cu ( $\mu\text{g/dL}$ ), Mn ( $\mu\text{g/L}$ ), and Zn (mg/L) levels were determined (Table 3). Selenium was strongly correlated with Cu ( $r=0.42$ ,  $p < 0.0001$ ) and Zn ( $r=0.27$ ,  $p < 0.001$ ).

A positive association was seen between Zn and TSH levels ( $r=0.12$ ,  $p < 0.05$ ). There were no significant correlations between free thyroid hormones (fT3 and fT4) and any trace element levels. For the thyroid auto-antibodies, an only positive correlation was detected between Mn levels and anti-TPO ( $r=0.14$ ,  $p < 0.05$ ) and Mn levels and anti-Tg ( $r=0.28$ ,  $p < 0.001$ ).

### Association between newborn screening-TSH and maternal trace elements

For the 315 newborns, the mean ( $\pm\text{SD}$ ) screening-TSH levels were found as 4.98  $\mu\text{U/mL}$  ( $\pm 3.50$ ). Newborn screening-TSH was positively correlated with maternal Cu ( $r=0.14$ ,  $p < 0.01$ ) (Figure 1). However, based on the high ( $>5 \mu\text{U/mL}$ ) and normal

**Table 1:** Analytical Conditions for zinc (Zn) and copper (Cu) measurement using flame atomic absorption spectroscopy (FAAS).

Element	Wavelength (nm)	Slit width (nm)	Lamp Current (mA)	Limit of detection (LOD) (mg/L)	Limit of quantification (LOQ) (mg/L)
Zn	219.9	0.7	8	0.005	0.014
Cu	324.8	0.7	6	0.0005	0.0006

**Table 2:** Subject demographics, maternal thyroid function test results and newborn screening-thyroid-stimulating hormone (TSH) (n=315).

Variable	Mean	SD	25th percentile	Median	75th percentile
Age	26.70	5.50	23.00	26.00	30.00
BMI <sup>a</sup> (kg/m <sup>2</sup> )	29.07	4.67	25.80	28.76	32.00
TSH (μU/mL)	2.59	1.94	1.40	2.12	3.10
ft3 (pmol/L)	4.44	0.65	4.00	4.43	4.80
ft4 (pmol/L)	11.97	1.93	10.70	11.97	13.20
AntiTPO (IU/mL)	11.84	27.80	5.00	6.02	8.40
Anti TG (IU/mL)	25.14	38.37	12.20	17.46	21.40
Newborn birth weight (g)	3204	502	2960	3160	3520
Newborn TSH <sup>b</sup> (μU/mL)	4.98	3.49	2.50	4.10	6.10

<sup>a</sup>Normal weight: 18.50 < BMI < 24.90, overweight: 25.00 < BMI < 29.90, and obese: BMI ≥ 30.0 [34].

<sup>b</sup>Newborn TSH was measured using the heel-prick blood that was obtained between 48–72 h after delivery. For maternal TSH (μU/mL) measurement, the reference intervals were 0.25–4.550 μU/mL, inter assay variation of the test was 3.62–4.28 and total coefficient of variation CV (%) was 5.13–6.64. For ft3 (pmol/L) measurement, the reference intervals were 3.50–6.50, inter assay variation of the test was 2.47–3.08, total CV (%) was 2.76–4.05. For ft4 (pmol/L) measurement, the reference intervals were 11.50–22.70, interassay variation of the test was 2.33–4.00, total CV (%) was 3.44–4.58.

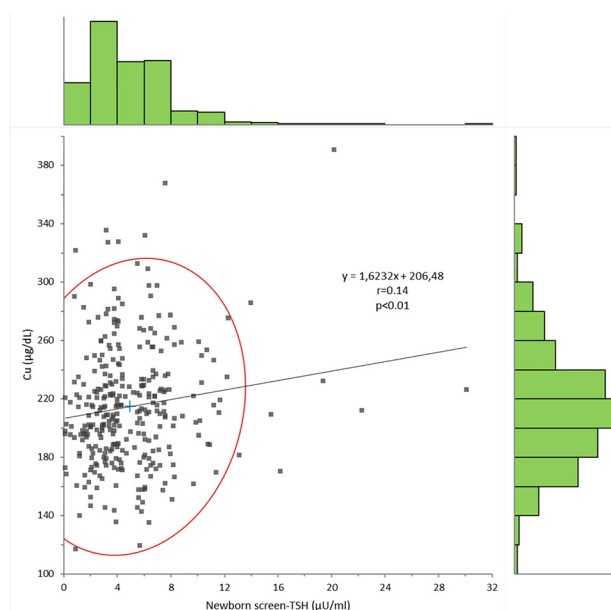
**Table 3:** Levels of maternal serum trace elements (n=315).

Element	Mean	SD	Minimum	Median	Maximum
Se (μg/L)	46.81	10.52	21.28	45.16	79.04
Cu (μg/dL)	214.54	41.41	117.04	210.10	390.64
Mn (μg/L)	2.11	0.57	0.20	2.11	3.46
Zn (mg/L)	0.44	0.08	0.24	0.43	0.66

(<5 μU/mL) levels of newborn screening-TSH, there was no statistical difference in maternal Cu levels ( $p > 0.05$ ) (Figure 2). Additionally, there were no significant correlations between newborn screening-TSH and any maternal trace elements.

### Multivariate analysis of newborn screening-TSH Levels and maternal trace elements

The relationship between the trace elements and newborn thyroid hormone levels was evaluated by a multivariate analysis which included clustering analysis using single linkage and multiple regressions.



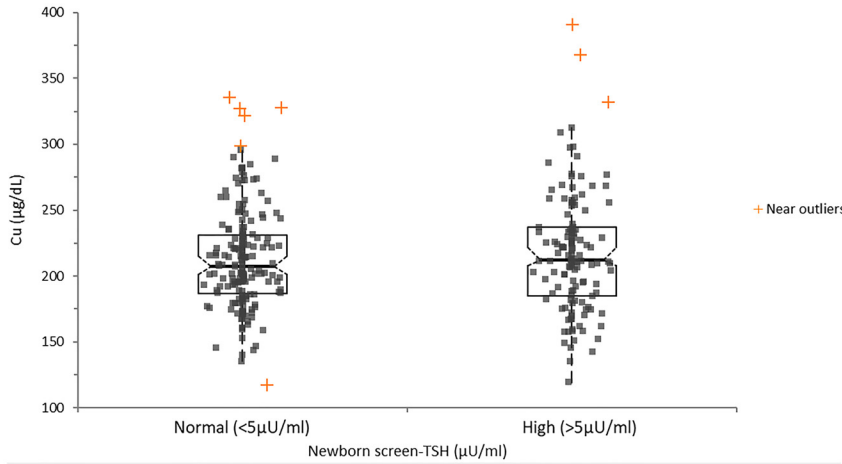
**Figure 1:** Correlation between maternal Cu and newborn screening-thyroid-stimulating hormone (TSH) levels (n=315). Pearson's correlation coefficient was determined as 0.14 ( $p < 0.01$ ). The frequency histogram represents the number of values (n) in the corresponding axis. The red curve shows 95% CI in the distribution.

In clustering analysis using single linkage, newborn screening-TSH and maternal TSH shared common clusters with Se, Mn, and Zn. An interesting finding of cluster analysis was that Cu exhibited a weak association with other variables, thus evidencing an imbalance of Cu levels in newborn and maternal thyroid health status (Figure 3).

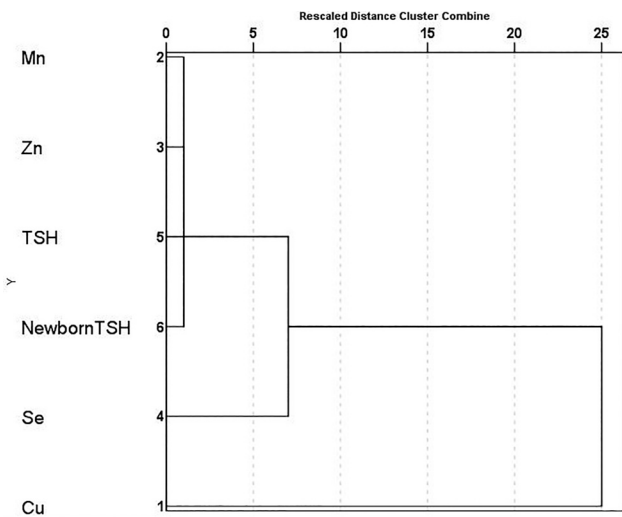
To test the interrelationship among the trace elements and newborn screening-TSH levels, we generated a multivariate linear regression model. Table 4 reports the results of this analysis of newborn screening-thyroid hormone levels on maternal trace elements and maternal TSH levels and their interactions. Multiple regression analysis of newborn screening-TSH levels controlling for maternal trace elements and maternal TSH levels showed a low but significant difference in the generated model ( $r=0.20$ ,  $p < 0.05$ ). From the model, we found that maternal Cu level was significantly associated with an increase in newborn screening-TSH levels ( $\beta=0.168$ ,  $p < 0.01$ ). No difference in newborn screening-TSH levels was observed for the rest of the maternal trace element levels.

## Discussion

The proper functioning and homeostasis of the thyroid gland are sensitive and vulnerable to the trace element levels. This is especially important as far as sensitive populations such as lactating women and their newborns



**Figure 2:** Relationship between maternal copper (Cu) levels and newborn screening-TSH levels (n=315). Based on the high (>5 µU/mL) (n=185) and normal (<5 µU/mL) (n=130) levels of newborn screening-TSH, there was no statistical difference in maternal Cu levels (p > 0.05).



**Figure 3:** Clustering analysis using a single linkage.

**Table 4:** Multivariate analysis of the effect of maternal serum trace elements (n=315) and maternal TSH (n=315) on the levels on newborn screening-TSH (n=315).

Variable	B	SEM (B)	t	Significance
Cu (µg/dL)	0.01	0.01	2.70	0.001
Mn (µg/L)	-0.56	0.34	-1.64	0.10
Zn (mg/L)	-3.21	2.49	-1.29	0.19
Se (µg/L)	-0.01	0.02	0.43	0.67
Maternal TSH	-1.21	0.10	-1.17	0.24

are concerned. Trace elements are ubiquitous in Turkish lactating women and all of them are detected within the reference ranges. Additionally, the present study represents the first assessment of maternal trace element levels in lactating women and its association with newborn screening-TSH levels in Turkish populations. Thus, our

data indicate that newborn screening-TSH levels are weakly affected by maternal trace element levels.

Copper is an essential trace element that has roles in growth and development. It acts as a co-factor for several vital enzymes participating in oxidative phosphorylation, detoxification of ROS, synthesis of neurotransmitters, and transport of iron [12–14]. We found that maternal serum Cu levels associate positively with newborn screening-TSH levels ( $r=0.14$ ,  $p < 0.01$ ), while other maternal trace elements not to be related to newborn screening-TSH levels. This positive association of maternal Cu levels and newborn screening-TSH levels may draw attention to the maternal trace elements that can have an impact on newborn thyroid status. It was previously shown that serum Cu levels are positively regulated by thyroid hormones, mainly stimulating the production of Cu-transport protein, ceruloplasmin [15]. Similarly, in a group of children diagnosed with congenital hypothyroidism, Cu exhibited a strong correlation with thyroid hormones [16]. The authors concluded that serum Cu and thyroid hormones are linked already in early postnatal life. In a study by Rossipal et al. the barrier function of the placenta was assessed and an active transfer of Mn and Zn, and diminished transfer of Se and Cu from maternal sera to umbilical cord sera were determined [17]. Interestingly, our data highlight that only serum Cu exhibited weak associations with the other trace elements and maternal and newborn screening-TSH in multivariate analysis. This pointed out that only maternal Cu can have a different effect on newborn thyroid health status. Additionally, the amount of Cu absorbed by a newborn is controlled by the ceruloplasmin gene expression in the mammary gland cells and alternative splicing of ceruloplasmin mRNA in the milk [18]. In the early days of postnatal development, breastfed newborns will have a natural control of copper balance in their bodies. Therefore, it can be deduced that maternal Cu

through breastfeeding has an impact on the postnatal development of the newborn, including thyroid homeostasis. In future studies, it will be valuable to gain deeper insights regarding ceruloplasmin levels and Cu functioning in pregnant and lactating women to associate the potential imbalance in Cu levels detected in the aforementioned population.

Zinc, another important trace element, is required for normal growth, development, reproduction, and immune function [19]. It is further known to act on the thyroid gland by effecting its morphology and metabolism [20]. For example, in one study animals fed with foods devoid of Zn had decreased hepatic type 5'-deiodinase, which resulted in the hampered conversion of T4 to T3 [21]. Additionally, Zn has antioxidant properties [21]. During thyroid hormone synthesis, a substantial amount of hydrogen peroxide is generated [22–24]. Considering the antioxidant properties of Zn, the positive association we found between maternal serum Zn and maternal TSH could be an indicator of a potential disturbance in the antioxidant mechanism in Turkish lactating women. It is known that even very low concentrations of neutral salts can strongly inhibit the binding of TSH to the thyroid plasma membrane. Neutral salts of Zn can contribute to a decrease in the binding of TSH to the thyroid plasma membrane [9]. Therefore, it might cause a positive association between serum Zn and maternal TSH levels ( $r=0.12$ ,  $p < 0.05$ ).

Selenium is very well known for its antioxidant properties and its relevance to thyroid autoimmunity [25]. However, we found no association between maternal Se levels and any maternal thyroid function test results. This can in part be explained by a high thyroid gland concentration of Se (0.2–2 µg/g), resulting in less amount of Se in the serum to establish any relationship with thyroid function test results [26–28]. Interestingly, we found a positive association between maternal serum Mn levels and thyroid auto-antibodies (anti-TPO and anti-Tg) ( $r=0.14$ ,  $p < 0.05$ ;  $r=0.28$ ,  $p < 0.001$ , respectively). One possible explanation for this association is that alterations in manganese superoxide dismutase, a major ROS detoxifier enzyme, in thyroid tissues are linked with thyroid dysfunction [9, 29, 30]. Therefore, Mn dependent antioxidant properties could affect immune-related thyroid auto-antibodies in lactating women.

In the literature, two major studies investigated the levels of trace elements in Turkish pregnant women [31, 32]. In one of the cited studies; folic acid, Zn, and Vitamin B12 levels were determined in Turkish pregnant women at 5–24 week of gestation [31]. The authors determined the mean Zn levels (0.8 mg/dl in patients in 1st trimester ( $n=145$ ), 0.75 mg/dl in patients in 2nd trimester ( $n=145$ )) almost twice as much as the levels that we found in our study

(mean Zn Levels: 0.44 mg/dl ( $n=145$ )). This might be due to the lack of Zn supplementation in our study group. In another study, serum Se, Zn, and Cu levels were investigated in pregnant women at 10–20 wk of gestation [32]. The mean Se levels in the cited study (44.85 µg/dL in patients in 1st trimester ( $n=177$ ), 47.18 µg/dL in patients in 2nd trimester ( $n=174$ ), 55.38 µg/dL ( $n=30$ ) in non-pregnant patients) and our study (46.81 µg/dL ( $n=315$ )) were found to be similar. However, there seems to be a dramatic increase in the serum Cu levels in different phases of gestation [32]. For example, in the same study the mean Cu levels were determined 104.75 µg/dL in non-pregnant patients and 132.33 µg/dL in patients' in the 1st trimester. However, in patients in the 2nd trimester, the mean Cu levels were found to be 164.86 µg/dL that indicating an increase in serum Cu levels [32]. In accordance with the cited study, we determined the mean Cu levels in the serum of women were found to be 214.54 µg/dL in the first 48 h after delivery. Interestingly, the multivariate analysis between maternal trace elements and newborn TSH showed that only maternal Cu exhibited a weak association with other variables, demonstrating a potential imbalance of Cu levels in newborn and maternal thyroid health status.

The high number of subjects and their newborns are a particular strength of this study, providing novel data regarding the potential effect of maternal trace element levels on newborn screening-TSH levels. One limitation of our study is that iodine concentrations were not included in the analysis. The maternal iodine concentrations substantially varied between lactating women (data not shown). The reason for this variation might be explained by the usage of iodine-containing disinfectants for preparing the skin before delivery. We believed different cutaneous absorption of iodine resulted in the substantial variations of maternal iodine concentrations. A major limitation of our study is that newborn screening-TSH levels were measured between 48 and 72 h after delivery. The blood samples obtained 3 to 5 days after birth will minimize false-positive high TSH levels caused by a surge in newborn TSH [33]. However, there was no statistical difference between the number of newborns with high ( $>5$  µU/mL) and normal ( $<5$  µU/mL) levels of newborn screening-TSH any maternal trace element levels and any maternal trace element levels. Newborn TSH analysis from heel-prick blood is routinely performed as part of a screening program, but it would have been a better comparison to take into account of newborn serum TSH levels. To make a general impression regarding the effect of maternal trace elements in newborn thyroid health status, other toxic and/or essential trace elements should have been measured in the maternal serum samples.

## Conclusion

In conclusion, there was no significant association between most of the maternal serum trace elements and thyroid hormone parameters in Turkish lactating women. Only significant associations were detected between serum Zn and TSH levels; and serum Mn and thyroid auto-antibodies (anti-TPO, anti-Tg). Additionally, the association between maternal serum Cu levels and newborn screening-TSH levels may highlight the importance of maternal trace elements on newborn thyroid status. Due to the critical roles of trace elements in thyroid functioning, the findings on the thyroid health status of lactating women and newborns are substantial.

**Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Informed consent:** Informed consent was obtained from all individuals included in this study.

**Author contributions:** All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Conflict of Interest:** The authors declare no potential conflicts of interest.

**Ethical approval:** All subjects gave informed consent and this study was approved by the Acibadem University Ethics Committee (ATADEK 2013-507).

**Çıkar çatışması beyanı:** Yazarlar tarafından potansiyel çıkar çatışması bildirilmedi.

## References

1. Agrawal P, Philip R, Saran S, Gutch M, Razi MS, Agroiya P, et al. Congenital hypothyroidism. *Indian J Endocrinol Metab* 2015;19:221–7.
2. Bost M, Houdart S, Oberli M, Kalonji E, Huneau JF, Margaritis I. Dietary copper and human health: Current evidence and unresolved issues. *J Trace Elem Med Biol* 2016;35:107–15.
3. Kohrle J, Jakob F, Contempre B, Dumont JE. Selenium, the thyroid, and the endocrine system. *Endocr Rev* 2005;26:944–84.
4. Jordan D, Suck C, Veisseire M, Chazot G. Zinc may play a role in the regulation of thyrotropin function. *Horm Res* 1986;24:263–8.
5. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* 2014;94:355–82.
6. Pasupathi P, Chandrasekar V, Kumar US. Evaluation of oxidative stress, enzymatic and non-enzymatic antioxidants and metabolic thyroid hormone status in patients with diabetes mellitus. *Diabetes Metab Syndr Clin Res Rev* 2009;3:160–5.
7. Karimi F, Omrani GR. Effects of selenium and vitamin C on the serum level of antithyroid peroxidase antibody in patients with autoimmune thyroiditis. *J Endocrinol Invest* 2019;42:481–7.
8. Koch KA, Pena MM, Thiele DJ. Copper-binding motifs in catalysis, transport, detoxification and signaling. *Chem Biol* 1997;4:549–60.
9. Soldin OP, Aschner M. Effects of manganese on thyroid hormone homeostasis: potential links. *Neurotoxicology* 2007;28:951–6.
10. Lain S, Trumpff C, Grosse SD, Olivieri A, Van Vliet G. Are lower TSH cutoffs in neonatal screening for congenital hypothyroidism warranted?. *Eur J Endocrinol* 2017;177:D1–D12.
11. Büyükgebiz A. Newborn screening for congenital hypothyroidism. *J. Clin Res Pediatr Endocrinol* 2013;5(2 Suppl):8–12.
12. Rubino JT, Franz KJ. Coordination chemistry of copper proteins: how nature handles a toxic cargo for essential function. *J Inorg Biochem* 2012;107:129–43.
13. Frieden E. Perspectives on copper biochemistry. *Clin Physiol Biochem* 1986;4:11–9.
14. Tapiero H, Townsend DM, Tew KD. Trace elements in human physiology and pathology. Copper. *Biomed Pharmacother* 2003;57:386–98.
15. Mittag J, Behrends T, Nordstrom K, Anselmo J, Vennstrom B, Schomburg L. Serum copper as a novel biomarker for resistance to thyroid hormone. *Biochem J* 2012;443:103–9.
16. Blasig S, Kuhnen P, Schuette A, Blankenstein O, Mittag J, Schomburg L. Positive correlation of thyroid hormones and serum copper in children with congenital hypothyroidism. *J Trace Elem Med Biol* 2016;37:90–5.
17. Rossipal E, Krachler M, Li F, Micetic-Turk D. Investigation of the transport of trace elements across barriers in humans: studies of placental and mammary transfer. *Acta Paediatr* 2000;89:1190–5.
18. Puchkova L, Babich P, Zatulovskaia Y, Ilyechova E, Di Sole F. Copper metabolism of newborns is adapted to milk ceruloplasmin as a nutritive source of copper: overview of the current data. *Nutrients* 2018;10:1591.
19. Majewska U, Braziewicz J, Banas D, Kubala-Kukus A, Kucharzewski M., Waler J, et al. Zn concentration in thyroid tissue and whole blood of women with different diseases of thyroid. *Biol Trace Elem Res* 2001;80:193–9.
20. Ruz M, Codoceo J, Galgani J, Munoz L, Gras N, Muzzo S, et al. Single and multiple selenium-zinc-iodine deficiencies affect rat thyroid metabolism and ultrastructure. *J Nutr* 1999;129:174–80.
21. Kralik A, Eder K, Kirchgessner M. Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. *Horm Metab Res* 1996;28:223–6.
22. Landex NL, Thomsen J, Kayser L. Methimazole increases H<sub>2</sub>O<sub>2</sub> toxicity in human thyroid epithelial cells. *Acta Histochem* 2006;108:431–9.
23. Sugawara M., Sugawara Y, Wen K, Giulivi C. Generation of oxygen free radicals in thyroid cells and inhibition of thyroid peroxidase. *Exp Biol Med (Maywood)* 2002;227:141–6.
24. Song Y, Driessens N, Costa M, De Deken X, Detours V, Corvilain B, et al. Roles of hydrogen peroxide in thyroid physiology and disease. *J Clin Endocrinol Metab* 2007;92:3764–73.
25. Negro R. Selenium and thyroid autoimmunity. *Biologics* 2008;2:265–73.
26. Ventura M, Melo M, Carrilho F. Selenium and thyroid disease: from pathophysiology to treatment. *Int J Endocrinol* 2017;2017:1297658.
27. Rayman MP. Selenium and human health. *Lancet (London, England)* 2012;379:1256–68.

28. Duntas LH, Benvenega S. Selenium: an element for life. *Endocrine* 2015;48:756–75.
29. Holley AK, Bakthavatchalu V, Velez-Roman JM, St Clair DK. Manganese superoxide dismutase: guardian of the powerhouse. *Int J Mol Sci* 2011;12:7114–62.
30. Nishida S, Nakano T, Kimoto S, Kusunoki T, Suzuki K, Taniguchi N, et al. Induction of manganese superoxide dismutase by thyroid stimulating hormone in rat thyroid cells. *FEBS Lett* 1997;416:69–71.
31. Erdogan M, Üstüner I, Cengiz B, Söylemez F, Çavdar AO. Effects of nutrition on zinc, folic acid, and vitamin B12 levels during pregnancy. *Biol Trace Elem Res* 2006;109:105.
32. Kilinc M, Coskun A, Bilge F, Imrek SS, Atli Y. Serum reference levels of selenium, zinc and copper in healthy pregnant women at a prenatal screening program in southeastern mediterranean region of Turkey. *J Trace Elem Med Biol* 2010;24:152–6.
33. American Academy of Pediatrics. Newborn screening for congenital hypothyroidism: recommended guidelines. *Pediatrics* 1993;91:1203–1209.
34. Centers for Disease Control and Prevention. Body mass index (BMI). Centers for Disease Control and Prevention, Atlanta, Georgia; 2017. Available from: <https://www.cdc.gov/healthyweight/assessing/bmi/index.html>.