Original article:

Correlation of Dietary Zinc Intake and Serum Zinc with Thyroid Stimulating Hormone (TSH) and Free Thyroxine (FT4) Levels in Adult Hyperthyroid Patients

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Abstract

Background: Hyperthyroidism is a thyroid disorder caused by increased synthesis and secretion of thyroid hormone by the thyroid gland. Hyperthyroidism is characterized by low thyroid stimulating hormone (TSH) and high free thyroxine (FT4) levels. The prevalence of hyperthyroidism in Indonesia was 0.6% in female and 0.2% in male. Zinc acts as an enzyme cofactor that converts thyroxine into triiodothyronine and it affects TSH synthesis. Adequacy of dietary zinc intake and serum zinc levels in hyperthyroid patients indirectly affects TSH and FT4 hormones. Objective: to analyze the correlation of dietary zinc intake and serum zinc with TSH and FT4 levels in adult hyperthyroid patients. Materials and Methods: This cross sectional study sampling based on inclusion and exclusion criteria which obtained 50 adult hyperthyroid patients at the Clinic of the Magelang Health Research and Development Center. Data of TSH and FT4 levels were measured by the ELISA method, serum zinc was measured with the ICP-MS method, dietary zinc intake’s data was collected using the 24 hour dietary recall and it was analyzed based on the table of Indonesian food composition 2017. Statistical analysis used in this study were spearman correlation and multivariate linear regression with 95% confidence level. Results: Dietary zinc intake did not correlate with both TSH and FT4 levels (p>0.05). Serum zinc had no correlation with TSH level (p>0.05), while serum zinc had a positive correlation with FT4 level (r=0.327; β=0.054; p<0.05). Conclusion: Serum zinc concentrations in adult hyperthyroid patients must be maintained because abnormal zinc status in the body’s metabolism will affect FT4 level. Keywords: dietary zinc intake, serum zinc, TSH, FT4, hyperthyroid.

Introduction

Hyperthyroidism is collection of clinical symptoms caused by excess thyroid hormones.¹ The most common cause of hyperthyroidism is Grave’s disease, an autoimmune disorder characterized by TSI (thyroid stimulating immunoglobulin) stimulate thyroid gland to increase thyroid hormones secretion. High levels of thyroxine (T4) as the main hormone of thyroid gland caused high levels of free thyroxine (FT4). TSI antibodies also bind TSH receptors in the thyroid gland that results in decreasing TSH levels.¹ ² The prevalence of hyperthyroidism was 1.2% in the United States population.³ The National Baseline Health Research of Indonesia in 2007 found 12.8% male and 14.7% female with low TSH levels,⁴ while based on the result of National Baseline Health Research 2013 there were 706,757 Indonesians aged ≥15 years who diagnosed with hyperthyroidism (0.4%). The prevalence of hyperthyroidism in Indonesia according to gender was 0.6% in women and 0.2% in men.⁵ Central Java Province had 0.5% of hyperthyroid population, higher than the national prevalence of Indonesia.⁶ Zinc is an essential mineral that needs in the body’s metabolism and plays a key role in the formation of thyroid hormones.⁷ Zinc is a cofactor

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of deiodinase enzymes which convert T4 to T3 and affect Thyrotropin releasing hormone (TRH) to stimulate TSH. Zinc deficiency can caused by low dietary zinc intake and inadequate zinc absorption in the intestinal tract. Study by Prasetyo et al. (2018) showed that 74.3% of Indonesian adult population tends to have low zinc intake.

Study in Pakistan showed that the mean level of serum zinc on hyperthyroid patients was lower than healthy subjects. Another study found that serum zinc level in hyperthyroid patients was higher than hypothyroid patients and zinc supplementation in hyperthyroid patients can prevent thyroid function disorders.

Adequate zinc intake and serum zinc levels in hyperthyroid patients indirectly affect thyroid hormones. Therefore, this study aimed to analyze the correlation between dietary zinc intake and serum zinc with TSH and FT4 levels in adult hyperthyroid patients.

**Materials and Methods**

This study had a cross-sectional design study which was conducted at the Clinic of Magelang Health Research and Development Center, it is a referral clinic for the management and treatment of thyroid disorders, including hyperthyroidism. This research protocol was approved by the Ethics Committee of the Medical Faculty, Sebelas Maret University, Surakarta, Indonesia (No. 014 / UN27.06 / KEPK / EC / 2020) and conducted from February-July 2020.

The population of this study was all adult hyperthyroid patients who have treatment at the Clinic of the Magelang Health Research and Development Center, there were 127 patients based on 2019 data. The sample was 50 patients which fulfilled inclusion and exclusion criteria. Inclusion criteria were hyperthyroid patients who are19-59 years old, TSH levels <0.3 mIU/mL, and patients with or without stroma. Exclusion criteria included hyperthyroid patients who were taking zinc supplementation, pregnant, menstruation, and patients with chronic disease or malignancy.

The independent variables were dietary zinc intake and serum levels. The dependent variables were TSH and FT4 levels. Phytate intake as a confounding variable. Dietary zinc and phytate intake was measured by the 24 hour dietary recall which interviewed 2 times on different days. Serum zinc levels were obtained using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. TSH and FT4 levels were measured by the ELISA method.

Data were collected after subjects agreed to participate in this study and filled out the informed consent. Blood sample of 5 cc was taken from vein in the arm’s subject for serum zinc, TSH and FT4 levels analysis, then the researchers interviewed dietary intake and conducted anthropometric measurements on the subjects.

Data was analyzed using the SPSS version 21 program. Normality of data was verified by Kolmogorov-Smirnov test. The correlation of dietary zinc intake and serum zinc with TSH and FT4 levels was analyzed using Rank Spearman correlation and multivariate linear regression. There were several limitations of this study, such as the sample size was small and high differences of the sample size in male and female subjects.

**Results and Discussion**

**Characteristics of Subjects**

General characteristics of subjects in this study are presented in table 1. Subjects consisted of 41 female patients (82%) and 9 male patients (18%). The mean age of the subjects was 40.4 years old. Half of the subjects had normal nutritional status (52%). The mean score of BMI was 22.97 kg/m².

**TSH and FT4**

The average level of TSH was 0.14 mIU/mL. Most of the subjects had low TSH levels (84%) and the rest had normal TSH levels (16%). The mean level of FT4 was 2.49 µg/dL. Subjects with high FT4 levels were 40%, normal FT4 levels were 54%, and low FT4 levels were 6%.
Diagnosis of hyperthyroidism is determined by TSH levels which are used as screening for thyroid function. TSH levels less than 0.3 mIU/mL indicate hyperthyroidism. Low TSH and high FT4 levels indicate overt hyperthyroidism. Subclinical hyperthyroidism characterized by low TSH and normal FT4 levels. Normal levels of TSH and FT4 indicate euthyroid.17

Subjects in this research were 16% euthyroid, 36% overt hyperthyroidism, and 48% subclinical hyperthyroidism. Along 1-2 weeks before the study, euthyroid patients had low TSH levels from laboratory results. Hyperthyroidism is often called “remitting and relapsing disease” that means the disease often recovers and reoccurs. Only about “remitting and relapsing disease” that means the disease often recovers and reoccurs. Only about 40-50% cases of hyperthyroidism have complete remission.17

All subjects took thyrozol as medication, average intake of thyrozol was 7.1 mg/day. The mean intake of thyrozol in the euthyroid, overt hyperthyroidism, and subclinical hyperthyroidism groups were 3.77; 10; 6.04 mg/day, respectively.

**Dietary Zinc Intake**

Recommended Dietary Allowance (RDA) of zinc were 11 mg/day for male and 8 mg/day for female (19-59 years old).18 The average of dietary zinc intake was 6.23 mg/day and most of the subjects had zinc deficiency (78%). Subjects with subclinical hyperthyroidism had the highest dietary zinc intake i.e 6.56 mg/day, but it was still lower than RDA.

This study result was in accordance with the study of Prasetyo et al. which showed that dietary zinc intake of Indonesian adult population was lower than RDA i.e 4.9 mg/day. Dietary zinc intake in hyperthyroid patients of present study was higher than previous study (6.23 vs 4.9 mg/day). However, the prevalence of zinc deficiency in this study was higher compared to the previous study (78% vs 74.3%).11

**Phytate Intake**

Phytate is a phosphorus storage molecule present in plant food which has negative effect on zinc absorption.19 Phytate is a strong chelator of zinc. Phytate cannot be digested or absorbed in the intestinal tract, so zinc bound to the phytate also passes through the intestine, unabsorbed. Phytate have high natural content in seeds and legumes, while lower content of phytate are available

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*mean±SD or n(%); OH: Overt Hyperthyroidism; SH: Subclinical Hyperthyroidism; BMI: Body Mass Index; Adequate dietary zinc intake: Male ≥11 mg/day; Female ≥8 mg/day; Deficient dietary zinc intake: Male <11 mg/day; Female <8 mg/day.

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**Table 1. Subjects characteristic according to type of hyperthyroidism**

| Characteristic of Subject | Total (n=50) | OH (n=18) | SH (n=24) | Euthyroid (n=8) |
|---------------------------|-------------|----------|----------|-----------------|
| 1. Age (years)            | 40.4±10.07  | 35.6±10.39 | 43.9±8.64 | 40.5±10.09     |
| 2. Gender:                |             |          |          |                 |
| Male                      | 9 (18%)     | 3 (16.7%) | 5 (20.8%) | 1 (12.5%)       |
| Female                    | 41 (82%)    | 15 (83.3%)| 19 (79.2%)| 7 (87.5%)       |
| 3. Nutritional status:    |             |          |          |                 |
| BMI (kg/m²)               | 22.97±3.57  | 22.28±3.25 | 23.38±3.63 | 23.28±4.28     |
| Underweight (<18.5 kg/m²) | 3 (6%)      | 1 (5.6%)  | 1 (4.2%)  | 1 (12.5%)       |
| Normal (18.5-25.0 kg/m²)  | 26 (52%)    | 10 (55.6%)| 13 (54.2%)| 3 (37.5%)       |
| Overweight (>25-27 kg/m²) | 6 (12%)     | 2 (11%)   | 3 (12.5%) | 1 (12.5%)       |
| Obesity (27 kg/m²)        | 15 (30%)    | 5 (27.8%) | 7 (29.1%) | 3 (37.5%)       |
| **Dietary Intake Data**   |             |          |          |                 |
| 4. Dietary zinc intake (mg/day) | 6.23±3.47  | 6.18±2.62 | 6.56±4.18 | 5.35±2.97      |
| Adequate                  | 11 (22%)    | 4 (22.2%) | 5 (20.8%) | 2 (25%)         |
| Deficient                 | 39 (78%)    | 14 (77.8%)| 19 (79.2%)| 6 (75%)         |
| 5. Phytate intake (mg/day) | 808.09±500.68 | 829.58±554.02 | 842.39±525.8 | 656.81±263.74 |
| 6. Phytate/zinc molar ratio (mmol/day) | 13.84±6.23 | 14.07±6.93 | 13.64±5.85 | 13.92±6.51     |
| **Biochemical Data**      |             |          |          |                 |
| 7. Serum zinc (µg/dL)     | 85.1±14.71  | 91.61±11.97 | 77.58±10.73 | 93±20.36       |
| Low (<60 µg/dL)           | 2 (4%)      | 0         | 2 (8.3%)  | 0               |
| Normal (60-130 µg/dL)     | 47 (94%)    | 18 (100%) | 22 (91.7%)| 7 (87.5%)       |
| High (>130 µg/dL)         | 1 (2%)      | 0         | 1 (12.5%) | 0               |
| 8. TSH levels (mIU/mL)    | 0.14±0.24   | 0.04±0.02 | 0.05±0.04 | 0.62±0.29      |
| Low (<0.3 mIU/mL)         | 42 (84%)    | 18 (100%) | 24 (100%) | 0               |
| Normal (0.3 – 4.0 mIU/mL) | 8 (16%)     | 0         | 0         | 8 (100%)        |
| 9. FT4 levels (ng/dL)     | 2.49±2.05   | 4.65±2.02 | 1.17±0.34 | 1.61±0.63      |
| High (>2.0 ng/dL)         | 20 (40%)    | 18 (100%) | 0         | 2 (25%)         |
| Normal (0.8 – 2.0 ng/dL)  | 27 (54%)    | 0         | 20 (83.3%)| 6 (75%)         |
| Low (<0.8 ng/dL)          | 3 (6%)      | 0         | 4 (16.7%) | 0               |
| **Drug History**          |             |          |          |                 |
| 10. Thyrozol (mg/day)     | 7.10±3.74   | 10±2.97   | 6.04±3.03 | 3.77±2.89      |
in fruits, leaves, and vegetables. The average phytate intake by subjects was 808.09 mg/day. The group with the highest intake of phytate was the subclinical hyperthyroid i.e 842.39 mg/day.

Phytate: zinc molar ratio has been used to estimate the proportion of absorbable zinc, because the inhibitory effect of phytate on zinc absorption appears to follow a dose-dependent response. The category of zinc absorption rates based on phytate:zinc molar ratio are <5, 5-15, and >15 mmol / day represents high, medium, and low zinc absorption rates. The molar ratio of phytate: zinc in this study was 13.84 mmol/day, categorized as medium rate of zinc absorption.

**Serum Zinc**

Almost the entire subjects (94%) had normal serum zinc levels, mean levels of serum zinc was 85.1 µg/dL while normal levels of serum zinc was 60-130 µg/dL. The subclinical hyperthyroid group had lowest serum zinc level, while euthyroid group had highest serum zinc level, there were 77.58 and 93 µg/dL, respectively. This results were in accordance with previous study on 49 hyperthyroid patients showed that hyperthyroid subjects had lower serum zinc levels than healthy subjects (4.54 vs 7.94 µg/g). Based on United Kingdom National Diet and Nutrition Survey, serum zinc concentration had significant positive correlation with dietary zinc intakes, which assessed by 7 day weighed food records. Previous study in New Zealand showed that were significant negative correlations between serum zinc levels with phytate intake and phytate:zinc molar ratio. Intakes of dietary zinc and phytate affect serum zinc concentration.

**Correlation of dietary zinc intake and serum zinc with TSH and FT4**

Table 3. Multivariate analysis of dietary zinc intake, serum zinc, and phytate intake with TSH and FT4 levels in adult hyperthyroid patients

| Dependent Variable | Independent Variable | B     | β standardized | t      | p     |
|--------------------|----------------------|-------|----------------|--------|-------|
| Dietary zinc intake (mg/day) | TSH (mIU/mL) | 0.001 | 0.010 | 0.056 | 0.955 |
| FT4 (ng/dL) | 0.169 | 0.284 | 1.691 | 0.098 |
| Serum zinc (µg/dL) | TSH (mIU/mL) | 0.002 | 0.010 | 0.686 | 0.496 |
| FT4 (ng/dL) | 0.054 | 0.384 | 2.837 | 0.007* |
| Phytate intake (mg/day) | TSH (mIU/mL) | -5.3x10^-4 | -0.112 | -0.610 | 0.545 |
| FT4 (ng/dL) | 0.000 | -0.096 | -0.572 | 0.570 |

*Correlation is significant

Zinc plays a key role in thyroid hormone metabolism. Zinc modulating transcription factors in thyroid hormone synthesis, regulating deiodinase enzyme activity, and synthesizes TRH and TSH. TSH describes the action of thyroid hormones on thyrotropic cells and indicates the whole metabolic status of thyroid gland. A slight increase in thyroid hormones especially FT4, will decreased secretion of TSH about 10 times. Thyroxine (T4) is the main hormone produced by thyroid gland, it is 93% of the total thyroid hormone. T4 released in a bound form with thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin. FT4 is a free form of thyroxine circulating in the bloodstream. Although just 0.03-0.05% FT4 in blood circulation, FT4 valid to describe hyperthyroidism state because it does not bind to protein.

The results of *Rank-Spearman* analysis between dietary zinc intake and serum zinc as independent variables with TSH and FT4 levels as dependent variables can be seen in Table 2. Serum zinc had no correlation with TSH levels (p>0.05) but it had positive correlation with FT4 (r=0.33; p=0.02). Phytate intake as a confounding variable did not correlate with TSH and FT4 (p>0.05). Dietary zinc intake did not correlate with both TSH and FT4 (p>0.05). Zinc intake which is bound to phytate cannot be absorbed in the intestinal tract. Inhibitory effect of phytate on zinc absorption depends on the dose of phytate against zinc. Therefore, phytate: zinc molar ratio was used to estimate the amount of absorbable zinc. Dietary zinc intake in this study had medium absorption,
according to the molar ratio of phytate: zinc.

Correlation between zinc and thyroid hormone has been still controversial, so that further analysis was carried out in this study. Result of multivariate linear regression can be seen in Table 3.

Zinc is required in deiodinase enzymes, these enzymes involved in synthesis of TRH and TSH. However, it was contrary to the study of Brandao-Neto et al (2006) which showed no correlation between serum zinc and TSH levels in healthy male subjects, increased TSH levels did not change serum zinc concentrations in subjects of previous study. Results of previous study are consistent with this study result which showed serum zinc did not correlate with TSH levels in adult hyperthyroid subjects (r=0.15; β=0.002; p>0.05).

Multivariate analysis showed that there was a significant positive correlation between serum zinc and FT4 levels (β=0.054; p<0.05). This result has consistency with study by Jain (2014) which showed that serum zinc levels was correlated with FT4 levels in healthy male subjects (β =-0.00063; p<0.01). Based on animal study by Baltaci et al(2017), zinc supplementation can prevent abnormalities of thyroid hormone levels in rats with thyroid disorders. Zinc increases the production of thyroxine-binding protein that can change the levels of T4.

Zinc is an essential mineral needed in the function of I-5’deiodinase enzyme which converts T4 into the active form T3. Deiodinase enzymes act to remove iodine molecules from T4. In conditions of zinc deficiency, activity of type I-5’deiodinase enzyme and conversion of T4 to T3 are limited which result in increases of T4 and FT4 hormones.

Abnormal serum zinc concentration will affect FT4 level in adult hyperthyroid patients, that it’s important to maintain normal level of serum zinc. Hyperthyroid patients should choose animal foods as protein sources, such as liver, poultry, beef, fish, eggs, and milk as sources of zinc. Animal foods do not contain phytates which resulted in better absorption of zinc from diet.

**Conclusion**

Based on our research in adult hyperthyroid patients, serum zinc concentration had positive correlation with FT4 levels. Adult hyperthyroid patients who have a higher level of serum zinc than normal value had higher risk to have a high level of FT4. Adult hyperthyroid patients must be maintaining normal levels of serum zinc because it will affect FT4 level.

**Ethical Approval:**

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and was stated as ethical conduct with the issuance of Ethical Clearance Number 014/UN27.06/KEPK/EC/2020. Ethical clearance was approved by the Ethics Committee of Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia

**Conflict of Interest**

Authors state no conflict of interest.

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**Authors’ contribution**

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