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Original Article

Effect of zinc supplementation on the zinc level in serum and urine and their relation to thyroid hormone profile in male and female goitrous patients

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SUMMARY

Background & aims: Zinc (Zn) is an essential element involved in many basic biochemical reactions in thyroid. The aims of present study is to evaluate the Zn status in biological samples and thyroid hormones levels in 60 goitrous male (GMPs) and 72 female patients (GFPs), before and after 6 months treatment with Zn supplementation and compared with non-goitrous subjects of both genders ($M = 106$, $F = 120$) of age range 16–30 years.

Methods: The biological samples were analyzed for Zn concentration using flame atomic absorption spectrophotometer, following their microwave assisted acid digestion. Quality control for the methodology was established with certified samples and with those obtained by conventional wet acid digestion method on the same CRMs and real samples.

Results: The results showed that the significantly lower mean values of Zn in serum, while high level urine samples of GMPs and GFPs were observed as compared to control subjects ($p < 0.005$ and 0.007) respectively. The mean values of free triiodothyronine and thyroxin were found to be lower in goitrous patients of both genders than in the age matched healthy control ($p < 0.006$ and 0.002) respectively, in contrast high mean values of thyroid stimulating hormone were detected in GMPs and GFPs ($p < 0.009$).

Conclusion: It was observed that Zn status and serum thyroid hormone levels were improved in goitrous patients after six months treatment with Zn supplementation.

1. Introduction

Zinc (Zn) deficiency is a major overlapping public health concerns in developing countries, while the correlation of Zn deficiency with goitrous problems was limited. Zinc is required for the proper function of 1,5′-deiodinase, the enzyme required for the conversion of thyroxine to the more active form, triiodothyronine.[1] The research by Wada and King[2] was of the first to investigate the relationship between Zn status and thyroid hormone levels, and effect of Zn supplementation on thyroid hormone function. They reported a significant decrease in free triiodothyronine (FT3) and free thyroxine (FT4) levels during Zn deficiency in goitrous male (GMPs) and female (GFPs) patients. Therefore, Zn intakes may be associated with decreases in thyroid hormone levels in goitrous patients.[2]

Thyroid disorders are the second most common endocrinopathies found in human and in animal study. Normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones.[3] In addition to iodine, several other micronutrients are involved in thyroid hormone metabolism, but only a few studies have addressed the possibility that marginal micronutrient deficits may contribute to explain the alterations in thyroid function observed in advanced age.[4,5] The roles of Zn in thyroid function are less established but sub- or supra-optimal dietary intakes of Zn can adversely affect thyroid hormone metabolism.[6] It was investigated that Zn deficiency has a synergistic effect on goiter formation.[7]

Several hormones affect Zn metabolism at physiological and biochemical levels.[8] One of the most important hormones, whose

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interaction with Zn has been investigated, is the thyroid hormones.[9] Impairment of growth and some endocrine disorders is due to Zn deficiency.[10] The interaction of thyroid hormones with Zn has been investigated[11,12] and Zn deficiency may be considered as a stimulus for goiterogenesis.[13]

Determinations of Zn in human tissues and fluids are used to obtain information on nutritional status for diagnosis of diseases, indication of systemic intoxication and to obtain information on environmental exposure.[14,15] The determination of trace metals in biological samples requires the use of sensitive and selective techniques such as atomic absorption spectrometer. This technique needs solubilization of the analyte and complete or partial decomposition of the matrix using either convective systems or microwave ovens and dry ashing. The main advantage of microwave assisted samples pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors.[16]

However, to the best of our knowledge, no study has been performed to evaluate the Zn concentration in males and females with thyroid disease in Pakistan. In our country like other developing countries malnutrition is the main problem, and deficiency of essential micronutrient, such as iodine, Zn, and iron is very common among population of urban and rural areas.[17,18] Due to these reasons, the prevalence of different physiological disorders is very common in urban and rural population. The present study was carried out to investigate the concentrations of Zn in biological samples (serum and urine) of male and female goitrous patients before and after 6 months treatment with Zn supplementation. The other biochemical parameters such as, thyroid stimulating hormone (TSH), FT3 and FT4 were also evaluated. For comparative purpose, the same biological samples were also collected from age matched healthy controls subjects of both genders residing in same areas. The Zn was analyzed by flame atomic absorption spectrophotometer (FAAS), prior to microwave-induced acid digestion (MDM). The accuracy of the Zn determination was tested by simultaneously analyzing certified reference materials (CRM) and conventional digestion method (CDM) on the same CRMs and real samples.

2. Materials and methods

2.1. Reagents and glassware

All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). Nitric acid (HNO_3) $\approx$16 M and 30% hydrogen peroxide (H_2O_2) were used. Ultra-pure water was prepared by passing de-ionized water from a Milli-Q system (Bedford, USA) and was used throughout the study. Standard solution of Zn was prepared by dilution of certified standard solution (1000 mg/l, Fluka Kamica). For validating of the analytical technique, certified reference samples of human serum (RECIPE-8880,) and human urine (RECIPE-8886,) were obtained from RECIPE Chemicals + Instruments GmbH (Munich, Germany), were used. All glassware and polyethylene bottles were thoroughly washed and then soaked overnight in 2 mol/l nitric acid, and rinsed with ultra-pure water before use.

2.2. Apparatus

A Perkin–Elmer model A. Analyst 700 (Norwalk, CT, USA) flame atomic absorption spectrophotometer with deuterium background correction was used. The hollow cathode lamp of Zn was run under the conditions suggested by the manufacturer. A single element hollow cathode lamp was operated at 7.5 mA and spectral bandwidth of 0.7 nm. The analytical wavelength was set at 214 nm. A Pel (PMO 23) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the samples. Centrifugation was carried out to separate the supernatant from the sample extracts with a WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge; speeds range 0–6000 rpm. Acid-washed plastic (polypropylene) vessels were used for preparing and storing solutions.

2.3. Study design and pretreatment

An epidemiological cross-sectional study was conducted among goitrous patients ($n = 132$) of both genders, age ranged as 16–30 years recruited from the outpatient clinic of Nuclear Institute of Medicine and Radiotherapy (NIMRA) Jamshoro and age matched non-goitrous subjects ($n = 226$). This study was designed as a Zn placebo controlled supplementation in goitrous male and female patients. Before start of this study, all the normal and goitrous patients were informed through a consent form about the aim of the study and all agreed to participate and signed the form. A questionnaire was also administered to them in order to collect details concerning physical data, ethnic origin, health, dietary habit, age and consent. Physical examinations were performed in the basic health unit of NIMRA to measure participant's weight, height, blood pressure and biochemical data. A file of complete information and all the demographical data was compiled. Supplementation, not very different from the RDA levels,[19] was given 5 day/wk for 6 month to Zn deficient GMPs and GFPs at NIMRA. Most people in this study belong to northern areas depend on farming, with rice as the primary crop. Thyroid patients of both genders were supplemented for six month with Zn (30 mg/day).

The criteria for the collection of biological samples (serum and urine) of GMPs and GFPs were set that, prior to any treatment, they were not taking any mineral supplement during last 3 months. The normal male and female subjects, belonging to same socio-economic status and dietary habits, who were mostly the healthy family members of the patients not suffering from any goitrous problem, were selected as control subjects. The preliminary exclusion criteria for patients and controls were hypertension, alcoholism, smoking, diabetes, cardiovascular disease, taking of any vitamin and minerals that could affect oxidative parameters.

All the venous blood samples (3–5 ml) (including control group) were collected after 12 h fasting, using metal-free Safety Vacutainer blood collecting tubes (Becton Dickinson, Rutherford®, USA) between 9.30 and 11.00 AM. The blood samples were left standing for one hour; sera were separated at 2500 rpm centrifugation and preserved at $-20\,^{\circ}C$ until analysis.[20] While for the analysis of other biochemical parameters up to 5 ml blood samples from the same subjects was sampled by using metal-free Safety Vacutainer blood collecting tubes containing $>$1.5 mg K_2EDTA and send to the pathological laboratories of NIMRA.

Morning urine samples were collected in an acid-washed, decontaminated 100 ml polyethylene tubes (Kartell 1, Milan, Italy). During sampling sessions, the containers were wrapped in a clean polyethylene bag. Urine samples were acidified with ultra-pure concentrated HNO_3 (l% v/v) and kept at $-4\,^{\circ}C$. Prior to subsampling for analysis, the sample should be shaken vigorously for 1 min to ensure a homogeneous suspension.

2.4. Biochemical analysis

To assess thyroid function, FT4 and FT3 were measured by using radio immuno-assay (Gamma counter, Oakfield, England, SD-12, 2000). Thyroid stimulating hormone was measured by using immuno radiometric assay (Gamma counter, Oakfield, England, SD-12, 2000). In all patients, measurements of thyroid hormones were performed initially at the time of diagnosis and 6 months after