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Metabolic processes

Low zinc levels may contribute to gynecomastia in puberty

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ABSTRACT

This study aimed to determine whether there were any differences in trace element levels between adolescent boys with gynecomastia and control boys and to determine the correlations between the levels of trace elements and body mass index (BMI) and sex hormones. The pubertal gynecomastia group comprised of 41 patients (mean age = 13.2 ± 0.9 years), who were admitted to Hacettepe University İhsan Doğramacı Children's Hospital in Ankara. Control group comprised of 21 healthy male children. Analyses of trace element levels were performed atomic absorption spectrometry. The mean zinc level of control group was 101.33 ± 16.87 μg/dL and the mean zinc level of gynecomastia group was 81.36 ± 17,43 μg/dL (20% lower in gynecomastia patients, $p = 0.0001$). However, the mean copper and manganese levels of gynecomastia patients were not statistically different than the control group. There were significant positive correlations between plasma zinc and total testosterone levels in gynecomastia group ($r = 0.592$; $p < 0.05$). There was a significant negative correlation between plasma zinc levels and BMI ($r = -0.311$; $p < 0.05$). These results indicate that zinc deficiency might be one of the underlying factors of gynecomastia, the importance of which needs to be further elucidated.

1. Introduction

Gynecomastia, derived from Greek words 'cwme' (women) and 'larsor' (breast), is defined as the benign proliferation of glandular breast tissue. Literally, "gynecomastia" originally indicates the "female breast" as the expression 'andromastia' would be more correct [1]. It can be observed in two different forms: physiologic gynecomastia and non-physiologic gynecomastia. Both forms of gynecomastia usually occur when the estrogen-to-testosterone ratio in men is disrupted and this phenomenon causes glandular breast tissue proliferation [1].

In newborns, adolescents and older men, physiologic gynecomastia is commonly observed. One-half of adolescent males can experience "pubertal gynecomastia", with a typical onset at 13–14 years of age [1,2]. Conditions like increased tissue sensitivity to normal male levels of estrogen can be one of the underlying levels of pubertal gynecomastia. [3]. It is usually self-limited; however, it can be treated in order to reduce the physical discomfort and the emotional distress of the patient, particularly if he is young [2]. On the other hand, different chemicals, drugs, supplements as well as genetic conditions may lead to non-physiologic gynecomastia in adolescents. In addition, the increase in blood estradiol levels and lagging free testosterone production can also cause non-physiologic gynecomastia. This condition can be treated with an anti-estrogen, such as tamoxifen, or surgery (liposuction or mammoplasty) [2,3].

The adolescent population (age 10–19) in the word comprises about 19% of the total population, accounting for 1200 million people. Adolescents are a nutritionally vulnerable population. They might have different micronutrient deficiencies. As they are rapidly growing, they have higher nutritional intake. However, malnutrition prevalence can be higher in adolescents vs. adults and this may affect their growth and development. Besides, they mostly have inappropriate eating habits. Recent studies have emphasized the significance of micronutrients in enhancing full growth potential. It has been stated that 60–80% of adolescents suffer from micronutrient deficiencies globally [4]. Adequate dietary intake of copper (Cu), zinc (Zn) and manganese (Mn) during childhood and adolescent period is essential for normal growth and development [4].

Zinc is an essential trace element and it is exceptionally important for human health. Zinc is a substantial component of > 200 enzymes. Some of these enzymes have substantial roles in the synthesis of nucleic acids [5]. It plays vital roles in reproduction, sex hormone synthesis, sexual maturation, androgen metabolism. Moreover, it is important for

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the optimal biochemical and physiological functions [6]. Zinc-deficient diet was shown to induce hepatic aromatization of testosterone to estradiol in rats and this phenomenon causes decreases in the circulating testosterone levels and increases in estrogen levels [5,6]. Zinc deficiency affects about two billion people in both developed and developing countries and causes growth retardation, infection susceptibility, and diarrhea in children. Every year, zinc deficiency is suggested to contribute about 800,000 children deaths worldwide [5,6].

Copper is largely present in organic complexes, many of which are metalloproteins. These proteins mainly act as enzymes [7]. In human cells, copper-zinc superoxide dismutase (Cu,Zn-SOD, SOD1 protein) is an antioxidant enzyme that is present in compartments of the cell, including cytosol, nucleus, mitochondrial intermembrane space and peroxisomes. Its primary function is to decrease the steady-state concentration of superoxide [8,9]. Manganese (Mn) is also an important trace element for human health and it is absolutely essential for growth, development, metabolism, reproductive system, and the antioxidant system [10]. As a cofactor, manganese is broadly available in different classes of enzymes and Mn-superoxide dismutase (Mn-SOD) is present in eukaryotic mitochondria. Mn-SOD enzyme is probably one of the most ancient enzymes. Nearly all of the aerobic organisms use this enzyme overcome the toxic effects of superoxide [11].

There is not any study in literature identifying the relationship between biological trace element status and breast proliferation in adolescents. Therefore, this study was conducted to determine whether there are any differences in trace element levels between adolescent boys with pubertal gynecomastia and control boys and to correlate the levels of trace elements to body mass index (BMI) and sex hormones.

2. Materials and methods

2.1. Chemicals

All chemicals were analytical grade and were purchased from Sigma-Aldrich ((St Louis, MO) or Merck Co (Darmstadt, Germany). For the measurement of testosterone, estradiol, total T_3 (TT_3), total T (TT), free T (FT) and free T (FT) levels were analyzed by ILEX Medical System (Petach-Tikva, Israel) kits using an Abbott Architect i2000 immunoassay analyzer (Abbott Laboratories, Abbott Park, IL). Thyroid stimulating hormone (TSH) levels were measured by electro-chemiluminescence immunoassay (ECLIA) kits using ECLIA-IIS (Fujian, China). Sex hormone binding globulin (SHBG) levels were determined by using Cisbio (Codolet, France) kits using Bioscan Chameleon as a Liquid Scintillation Counter (Ed- munds, WA).

2.2. Subjects

The study was approved by Hacettepe University's ethical committee. Families filled out a standard questionnaire and parents and children before participation gave a written informed consent.

The groups in the study were as follows:

1. Control group (11.5–14.5 years old, mean age:13.2 ± 1.1 years): 21 healthy male children of with comparable age with no history of gynecomastia and any other endocrine disorder. 2. Gynecomastia group (11 and 15 years old; mean age: 13.2 ± 0.9 years): 41 physiologic, pubertal gynecomastia patients (Table 1). All patients were examined by the same pediatrician. Diagnosis of gynecomastia was made by the standard approach [12]. Age distribution was not different between the two groups.

Table 1
Age and body mass in index in study groups.

Group	Age (years)	BMI
Control Group (n = 21)	13.2 ± 1.1	20.6 ± 3.1
Gynecomastia Group (n = 41)	13.2 ± 0.9	19.9 ± 3.9

Results are given as mean ± SD (SD: Standard deviation).
BMI: body mass in index.

Between October and December 2007, the children were admitted to Hacettepe University İhsan Doğramacı Children's Hospital in Ankara. The BMI was calculated (weight divided by the square of height) and obese children (BMI percentile range ≥ 95%, according to Centers for Disease Control and Prevention, CDC) were not recruited to the study [13].

We have conducted a simple survey, in which we evaluated the food consumption, healthy diet and healthy life style of the subjects recruited to this study. None of the subjects in any of the study groups showed signs of malnutrition. All of the study subjects (other than presence of gynecomastia in the gynecomastia group) were healthy and received a well-balanced diet, containing bread, vegetables, fruits and meat (both white and red).

2.3. Sampling of blood

Venous blood samples were taken into heparinized tubes. Samples were centrifuged at 800 × *g*. After obtaining plasma, all samples were aliquoted and stored at −80 °C until analysis. Precaution was taken in both collection and subsequent handling of plasma samples in order to avoid trace element contamination.

2.4. Measurement of zinc, copper and manganese levels by atomic absorption spectrometry

Zinc and copper were analyzed by using flame atomic absorption spectrometry (FAAS, Schimadzu, Japan). Analysis of manganese was performed by graphite furnace atomic absorption spectrometry (GFAAS, Schimadzu, Japan). Plasma samples were diluted by deionized water by a factor of 30. Stock solutions for zinc, copper and manganese were prepared as 1000 ppm by using twice distilled water. Standard solutions were prepared in the volume of 100 mL where 5 mL of hydrochloric acid was added. The basic set of standards for construction of analytical curves was prepared from these stock solutions. Standard solutions with concentrations 1–100 μg/dL were prepared for copper and zinc. The concentration interval for standard solutions was 0.1–1 μg/L for manganese. Optical densities were read at 324.8 nm, 213.9 nm, and 279.5 nm for copper, zinc and manganese, respectively. In order to obtain assay accuracy and higher quality, for every 10-test sample, the standard solutions were run. A software package (SpactrAA software) was used to calculate concentrations of the trace elements.

Blank plasma samples, spiked with levels of 50 μg/dL for copper and zinc, and 5 ng/mL for manganese, were used in recovery studies were performed. The average recoveries were found to be (mean ± SD) 94 ± 3.1% for zinc; 95 ± 2.4% for copper and 91 ± 4.2% for manganese on 30 occasions. Between-run precisions were 9.12 ± 1.14% coefficient of variation (CV) for copper, 11.04 ± 2.25% CV for zinc and 12.07 ± 1.91% CV for manganese. Within-day precisions were 11.25 ± 3.41% CV for copper, 10.14 ± 1.97% CV for zinc and 13.14 ± 4.14% CV for manganese. Limits of detection (LOD) for copper, zinc and manganese were 10 μg/dL, 10 μg/dL and 1 ng/mL, respectively.

2.5. Hormone measurements

Serum estradiol, TT, total T TT, FT and FT levels were measured by chemiluminescence microparticle immunoassay. TSH levels were determined by ECLIA. Serum total testosterone and dehydroepiandrosterone sulfate (DHEAS) levels were also measured by solid-phase chemiluminescence immunoassay. Sex hormone binding globulin (SHBG) levels were tested by immunoradiometric assay.