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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.

2.6. Statistical analysis

The results were expressed as mean ± standard deviation (SD) by using Statistical Package for the Social Sciences (SPSS, Windows Version 17) software. Differences between groups were determined by independent sample *t*-test. Correlations were determined by using Pearson's correlation coefficient (*r*). $p < 0.05$ values were evaluated to be statistically significant.

3. Results

The BMI of the control group was 20.6 ± 3.1 while the BMI of the study group was 19.9 ± 3.9 and therefore both groups had comparable BMI levels (Table 1). The BMI-for age percentiles for control group were as follows: 10% of the children were in 1–24%, 50% of the children were in 25–75% and 40% of the children were in 76–94% of BMI. The BMI percentages for gynecomastia patients were as follows: 23.1% of the children were in 1–24%, 43.6% of the children were in 25–75% and 33.3% of the children were in 76–94% of BMI. 20% of the control children and 30.7% of the gynecomastia children had family history for pubertal gynecomastia. 12.2% of the gynecomastia patients had right breast gynecomastia while 12.2% had left breast gynecomastia. 75.6% of the gynecomastia patients had both breast gynecomastia. Total and free testosterone levels for gynecomastia patients were 168.22 ± 137.35 ng/dL (mean ± SD) and 3.79 ± 2.81 ng/mL (mean ± SD), respectively (mean ± SD). Estradiol levels for gynecomastia patients were 9.48 ± 9.55 pg/mL (mean ± SD). SHBG levels were 30.43 ± 13.77 nmol/Lwhile DHEAS levels were 144.87 ± 68.87 μg/dL; FT4 and FT3 levels were 15.16 ± 2.02 pmol/L and 6.51 ± 0.86 pmol/L, respectively. TT4 and TT3 levels of the patients were 8.53 ± 1.58 μg/dL and 1.70 ± 0.23 μg/dL, respectively [12].

All of the children in the gynecomastia group had normal cholesterol, HDL and LDL levels. Besides, none of the children had triglyceride levels higher than the normal ranges for children. The cholesterol levels were 129.30 ± 14.25 mg/dL while HDL levels were 68.28 ± 9.19 mg/dL and LDL levels were 82.24 ± 13.47 mg/dL in gynecomastia group. Serum triglyceride levels of gynecomastia patients were 78.19 ± 9.52 mg/dL.

Mean plasma/serum zinc level of humans was reported to be 60–130 μg/dL [14]. Therefore, the mean plasma zinc levels of both control and gynecomastia levels were in a normal range. Only two subjects from gynecomastia group had zinc deficiency (54 and 59 μg/dL). The mean zinc level of control group was 101.33 ± 16,87 μg/dL (min:78,1; max:131 μg/dL)and the mean zinc level of gynecomastia group was 81.36 ± 17.43 μg/dL (min:54; max:118 μg/dL). Zinc levels in gynecomastia group showed ~20% decrease in gynecomastia patients and were significantly lower than control ($p = 0.0001$) (Table 2).

Mean plasma/serum copper level of humans was reported to be 70–160 μg/dL [14]. Therefore, the plasma copper levels of both control and gynecomastia levels (65–141 μg/dL) were in normal range, except two subjects in gynecomastia group (65 and 66 μg/dL). The mean copper level of control group was 102.29 ± 9.98 ng/mL (min:85; max:124 ng/dL) and the mean copper level of gynecomastia group was 96.93 ± 14.06 μg/dL (min:65; max:141 ng/dL). In gynecomastia patients, copper levels showed 6% decrease and were not significantly different between than control group ($p = 0.125$) (Table 2).

Mean plasma/serum manganese level of humans was reported to be 4.7–18.3 ng/mL for humans [14]. Therefore, the manganese levels of both control and gynecomastia levels were in normal range. The mean manganese level of control group was 11.13 ± 1.81 ng/m (min:7.78; max:14.17 ng/mL) and the mean manganese level of gynecomastia group was 10.50 ± 1.86 ng/mL (min:6.54; max:15.14 ng/mL). Manganese levels showed 6% decrease in gynecomastia patients and were not markedly different than control group ($p = 0.209$) (Table 2).

There were no significant correlations between breast size scores of gynecomastia groups and trace elements. However, there were significant positive correlations between plasma zinc levels and total testosterone ($r = 0.592$) and SHBG ($r = 0.305$) in gynecomastia group ($p < 0.05$, both) (Table 3). Besides, there were negative significant correlations between plasma zinc levels and BMI ($r = -0.391$), estradiol ($r = -0.311$) and DHEAS ($r = -0.304$) ($p < 0.05$, all). Moreover, plasma cholesterol, LDL and triglyceride levels were negatively correlated with plasma zinc levels for the gynecomastia group ($r = -0.366$; $r = 0.287$; and $r = -0.345$, respectively; $p < 0.05$ for cholesterol and triglyceride). On the other hand, plasma HDL levels were positively correlated with plasma zinc levels ($r = -0.312$, $p < 0.05$). Plasma copper levels were positively correlated with estradiol ($r = 0.388$) and TSH ($r = 0.453$) ($p < 0.05$, both). Besides, plasma manganese levels were inversely correlated with TT4 ($r = -0.302$) and TSH ($r = -0.316$) and positively correlated with SHBG ($r = 0.297$) ($p < 0.05$, all). No significant correlations were found between plasma lipid profiles vs. plasma copper levels as well as vs. plasma manganese levels in gynecomastia patients.

Table 2
Zinc, copper and manganese levels in study groups.

Group	Zinc (μg/dL)	Copper (μg/dL)	Manganese (ng/mL)
Control Group (n = 21)	101.33 ± 16.87 (min:78.1; max:131)	102.29 ± 9.98 (min:85; max:124)	11.13 ± 1.81 (min:7.78; max:14.17)
Gynecomastia Group (n = 41)	81.36 ± 17.43[a] (min:54; max:118)	96.93 ± 14.06 (min: 65; max: 141)	10.50 ± 1.86 (min:6.54; max:15.14)
	(p = 0.0001)	(p = 0.125)	(p = 0.209)
One breast gynecomastia (n = 10)	84.26 ± 17.80[a] (min:65; max:118)	104.30 ± 14.83 (min: 84; max: 112)	10.01 ± 1.71 (min:7.30; max:12.56)
	(p = 0.014)	(p = 0.657)	(p = 0.112)
Two breast gynecomastia (n = 31)	80.94 ± 17.30[a] (p = 0.0001)	96.00 ± 15.35 (min: 65; max: 141)	10.64 ± 1.98 (min:6.54; max:15.14)
	(min:54; max:114)	(p = 0.103)	(p = 0.374)

Results are given as mean ± SD (SD: Standard deviation).
[a] Significantly different than control.

Table 3
Pearson's correlation coefficients (*r*) between breast size scores, plasma hormone levels, BMIs, plasma lipid profile and trace element levels of gynecomastia patients.

Parameter	Zinc *r*	Copper *r*	Manganese *r*
Left-breast gynecomastia	−0.143	−0.298*	−0.11
Right-breast gynecomastia	−0.160	−0.201	−0.04
Total Testosterone	0.592*	−0.065	−0.157
Estradiol	−0.311*	0.388*	0.048
TT_3	−0.171	−0.185	0.048
TT_4	−0.002	0.037	−0.302*
FT_3	−0.003	−0.112	0.049
FT_4	−0.003	0.194	−0.065
TSH	−0.169	0.453*	−0.316*
SHBG	0.305*	0.198	0.297*
DHEAS	0.304*	0.073	0.116
BMI	−0.391*	−0.240	−0.06
Cholesterol	−0.366*	−0.177	−0.114
HDL	0.312*	0.112	0.087
LDL	−0.287	−0.118	0.111
Triglyceride	−0.345*	−0.101	0.137

BMI: body mass index.
* Significantly correlated ($p < 0.05$).

4. Discussion

The growth of the male glandular tissue can be induced by estrogens. Therefore, increased cellular levels of estrogen can be one of the

underlying causes of gynecomastia. Endogenous overproduction or exogenous supplementation of estrogen may lead to biologically increased levlesl of estrogen. Besides, increased biotransformation of androgens to estrogens can also lead to increased estrogen concentrations. Besides, inhibition of the degradation of estrogens might be the cause of excess levels of estrogen in the body. However, the role of androgens is less clear in the growth of male breast tissue. In normal and pathological male breast tissue, androgen receptors are present. Androgens clearly cause inhibition of breast tissue growth and in individuals with "androgen insensitivity syndrome", gynecomastia may be observed as one of the main phenotypic characteristics [2,10].

Zinc is the only trace element, which is available in the structure of almost all enzyme classes [5]. It is known that high concentration of zinc is present in testes and accessory sex glands and this phenomenon is an indicator of its substantial role in the function of male reproductive system [5]. Zinc is also crucial for the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and also for spermatogenesis, testicular steroidogenesis, androgen metabolism and interaction with steroid receptors [5,15]. The interaction between serum testosterone and plasma zinc concentrations has been shown before by several animal studies [16].

Zinc deficiency is commonly seen throughout the world, including the USA. In men, hypogonadism may be caused by severe or moderate zinc deficiency [5,17]. Zinc is an essential trace element for the synthesis and secretion of several hormones, including LH and FSH. Besides, zinc affects differentiation of gonads, Müllerian inhibiting factor action, growth of testis and development of seminiferous tubules, spermatogenesis, testicular steroidogenesis, androgen metabolism and interacts with steroid receptors [16]. However, it is still not clear how the effect of serum testosterone concentration is affected from marginal zinc deficiency in humans. In humans, supplementation of zinc after marginal zinc deficiency provided increases in serum testosterone levels and it was suggested that zinc may be a crucial player in serum testosterone levels modulation in normal men [5].

Several animal studies have addressed the relationship between sex hormones and zinc. In zinc deficiency, rat testicular cells were able to take up precursors of steroids, sex steroids, namely cholesterol and neutral lipids; however, these cells became incapable of converting them into sex steroids, leading to the arrest of spermatogenesis and the impairment of fertilization [18]. It has been reported that the receptor proteins have a zinc binding component with a high affinity for testosterone and dihydrotestosterone (DHT) [5], and that androgen receptor sites are markedly lowered in the reproductive organs of zinc-deficient rats [19]. In the present study, although only two subjects in gynecomastia group were deficient in zinc, plasma zinc levels were significantly lower in gynecomastia patients compared to control group. Besides, a marked positive correlation between plasma zinc levels and plasma testosterone levels in gynecomastia patients was observed in the current study. Most circulating testosterone rapidly passes through the hepatocytes membranes and is converted to both estradiol (by aromatase) and to DHT subsequently into 3a-diol (by 5α-reductase). Zinc deficiency was shown to reduce circulating LH and testosterone concentrations, alters 5-α reduction and aromatization of testosterone in rats. 5α-reductase activity in liver was found to be lower in animals fed a zinc-deficient diet, emphasizing that zinc is a substantial element for hepatic 5α −reductase activity [20]. Furthermore, male rats fed with zinc-deficient diet showed a higher hepatic aromatization of testosterone to estradiol [19]. This is one of the mechanisms by which the levels of circulating testosterone are decreased and the level of estrogen is increased [20]. On the other hand, DHEAS levels were positively correlated with plasma zinc levels, the importance of which needs to be evaluated with future studies. Our assumption is that as DHEAS is the main source for testosterone production (through different reactions and finally by the conversion of estradiol to testosterone by aromatase), increase in plasma zinc levels might directly have an impact on DHEAS production and finally on testosterone synthesis. Therefore, we can postulate that decreases in plasma zinc levels might be one of the underlying factors of gynecomastia, as zinc has a very important role in aromatization of testosterone.

We observed a negative significant correlation between BMI and plasma zinc levels of the gynecomastia patients ($r = -0.391$). Tamura et al. observed that the pregnant subjects with higher BMI had significantly lower plasma Zn concentrations [21]. Zaky et al. found a significant negative correlation ($r = -0.453$, $p = 0.030$) in zinc and BMI levels in obese patients [22]. Di Martino et al. also determined that zinc levels in obese patients were significantly ($p < 0.01$) lower than in controls, whereas the BMI values were significantly greater [23]. Although our results are in accordance with the findings of the limited number of studies, there is not a proposed mechanism to explain this negative correlation. We can suggest that particularly obese people may not be receiving sufficient levels of Zn by their diet as they mainly consume carbohydrates, sugar and fat and zinc deficiency might be a possible outcome of their feeding habits. In the present study, we observed a negative correlation ($r = -0.240$, $p > 0.05$) between plasma copper concentrations and BMI in gynecomastia patients, confirming the recent findings. Blindauer et al. showed that zinc has a major binding site on albumin [32]. Barnett et al. suggested that albumin mediates crosstalk between zinc and fatty acids. They also hypothesized that zinc binding to site A (on albumin) and fatty acid binding to FA2 might be mutually exclusive, but it was unclear whether zinc would preclude fatty acid binding or vice versa. Interactive binding of zinc and longer chain fatty acids has potential physiological and clinical consequences. On the other hand, in a systematic review and meta-analysis, zinc supplementation significantly reduced total cholesterol, LDL, cholesterol and triglycerides [33]. Therefore, it was suggested that zinc may have the potential to reduce the incidence of atherosclerosis related morbidity and mortality. The cholesterol, LDL and triglyceride levels of the subjects in the two study groups were negatively correlated with zinc concentrations, while HDL levels were positively correlated with plasma zinc levels of gynecomastia patients as also suggested by Ranasinghe et al. [34].

Plasma/serum copper and ceruloplasmin levels are most often used to assess copper nutritional status [24]. In a recent study, the geometric mean copper levels of American population was found to be 125.1 (121–129.4) μg/dL which is ~20% higher than the current study [24]. Copper deficiency is not common among humans and only two subjects in gynecomastia group had copper deficiency in the current study. Besides, we did not observe a significant difference in plasma copper levels between gynecomastia and control groups.

The levels of copper and ceruloplasmin change depending on gender. However, the effects of sex hormones, particularly estradiols, and their relationship with factors that interfere with copper metabolism are not clear. Research has shown that estradiol increased cellular copper concentration and mRNA expressions of proteins responsible for copper uptake (hCTR1 and DMT1) and changed the expression patterns of these proteins [25]. Besides, estrogens significantly influence the cellular levels of copper and the responses of the whole body to copper. This phenomenon suggests that estrogens may help maintaining copper availability for metabolic needs [24]. Copper was also shown to potentiate the estradiol-induced response in a dose-dependent manner in yeast. Significant induction was obtained from at 100 nM copper [26]. In the present study, we observed a significant positive correlation between plasma copper and plasma estradiol levels ($r = 0.388$), verifying the past findings.

Copper, zinc, and selenium not only affect thyroid function but also interact and possibly affect one another. Serum copper levels are regulated by thyroid hormones, which stimulates the synthesis and the export of the hepatic copper-transport protein ceruloplasmin into the serum [27]. Jain observed that serum copper levels were associated with increased levels of FT_4. We also observed the same phenomenon in the present study. FT_4 levels of the gynecomastia patients were found to be positively correlated with the plasma copper levels ($r = 0.194$,

$p > 0.05$) [24]. In addition, we also observed that TSH levels of gynecomastia patients were also significantly and positively correlated with plasma copper levels ($r = 0.453$, $p < 0.05$).

Among the gynecomastia patients, there was a tendency for high BMI subjects to present lower plasma levels of copper. According to Hatano et al. [28], in obese subjects excess weight along with lipid metabolism disorders might predispose to alterations in plasma copper levels. This phenomenon is suggested to be more evident in males, indicating a possible mechanism of this mineral, contributing to peroxidation or acting as an antioxidant [28]. groups were in normal ranges.

Although to date there is no method stablished to evaluate the nutritional status of manganese among humans, the values in serum or plasma are commonly used [10]. In the current study, the mean manganese levels of both control and gynecomastia patients were in normal range and the manganese levels in gynecomastia group were not significantly different from control. Plasma manganese levels were found to be significantly and negatively correlated with plasma TT_4 and TSH levels. The metabolism of zinc, copper, manganese, and selenium is abnormal in thyroid diseases [29]. In rats, manganese treatment (as $MnSO_4$, 1 mg/100 g/day, s.c.) produced no change in thyroid T_4 and T_3 levels but induced a significant decrease in serum T_4, T_3 and TSH levels [30]. The serum levels of T_4, but not T_3, were slightly reduced by manganese treatment (as $MnCl_2$, 200 mg/L in drinking water) in female mouse [31]. There are limited number of studies that show the interaction between plasma manganese levels, thyroid hormones and sex hormones. Therefore, it is not possible to evaluate the interaction between manganese levels and the measured hormones currently. Mechanistic studies on a wider approach need to confirm the findings of the present study.

5. Conclusion

Most researchers address an 'imbalance of androgen and estrogen action' as a pathogenic factor in the etiology of gynecomastia. This imbalance can result from genetic factors or environmental agents. However, there may be other underlying factors that might include trace element status. Limited number of studies suggests that trace element levels, particularly zinc levels, are correlated with plasma/serum testosterone levels. We also suggest a positive correlation between plasma zinc and testosterone levels herein. Moreover, we have also found significant relations between trace elements levels and TSH, thyroid hormones, SHBG and DHEAS. However, there are only few reports suggesting the mechanism by which trace elements and these hormones interact with each other. Besides, there are no studies showing such interactions in endocrine diseases, like gynecomastia. This present work is the first study that manifests these interactions.

In conclusion, we can indicate that alterations in trace element status, specifically in zinc levels, can lead to different pathological conditions and can be one of the factors underlying gynecomastia. However, other disruptions or alterations in the hormonal status or in enzyme activities (that are responsible for the synthesis or conversion of sex hormones) should also be present along with the alterations in trace elements. However, larger studies are needed to show such interactions in different endocrine disorders. Besides, mechanistic studies are needed to bring a new approach to this interplay.

Conflict of interest

The authors declare that they have no competing interests.

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