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Estimation of Trace elements and Thyroid Hormones in thyroid disorders patientsin Kirkuk –Iraq

Noor Mohammed Kadhim[1], Kameran Shukur Hussain[1]
[1]*College of Science – The University of Kirkuk.*
***Corresponding authors:**no.or997@yahoo.com,Kamerandalo@uokirkuk.edu.iq

Abstract:
Background: Iraqis frequently suffer from thyroid conditions, and hypothyroidism has increased since the events of 2003 and 2014. Thyroid health and disease are linked to trace elements. The production and processing of thyroid hormones require various trace elements like zinc (Zn), magnesium (Mg), iron (Fe), iodine (I), selenium (Se), and copper (Cu) for the proper functioning of the thyroid gland. These trace elements are in a dynamic equilibrium and interact with one another. The excess or lack of one or more substances, however, has the potential to upset this equilibrium, resulting in aberrant thyroid function as well as the promotion of autoimmune thyroid disorders and thyroid malignancies.
Objectives:to assess the thyroid health of Kirkuk province residents and discover how it relates to the trace elements zinc, copper, magnesium, and iron.
Setting:100 patient from Ibn-Nafees private medical lab participated in a preliminary cross-sectional survey. with thyroid issues, of both sexes. They were 38.12 years old on average, plus or minus 1.4 years. **Methods:**The Mini-Vidas machine has been utilized to evaluate thyroid hormones T3, T4 , TSH, and Trace elements Zn, Cu, Mg, and Fe are measured by flame atomic absorption spectroscopy (FAAS) .
Results: In this study, high significant increase of TSH serum level (with p-value= 0.0001) was obtained in hypothyroidism patients, and high significant increase of T4 serum level (p-value= 0.0018) was obtained in patients with hyperthyroidism,in comparison to the control group.Patients who had thyroid disorders exhibited notable variations (with a p-value of $\leq$ 0.05) in the levels of elements; (Mg, Zn, Cu, and Fe). The findings indicated that individuals with hypothyroidism had lower levels of Zn and Mg compared to those with hyperthyroidism and a control group, whereas lowered levels of Fe and Cu in hypothyroidism patients and an increase in hyperthyroidism patients were noticed compared to healthy subjects.The relationship between TSH hormone and T4 hormone found to be inversed(with r= -0.35) in both hypothyroidism and hyperthyroidism patients. There exists a direct relationship between Copper and Zinc variables (with r= 0.41), and also a direct relationship between the variables Magnesium and Zinc (with r= 0.33) in patients with thyroid disorders.
Conclusions: Thyroid issues are extremely prevalent among Kirkukians. It was necessary to monitor trace elements including zinc, copper, magnesium, and iron in hypothyroid patients in order to rule out any anomalies. Further investigation involving more patients are needed to gain additional insights into the impact of thyroid dysfunction on human body and its potential association with trace element deficiencies.

Keywords: *Hyperthyroidism, hypothyroidism, Thyroid hormones, Iron (Fe), Zinc (Zn), Copper (Cu), Magnesium (Mg).*

Introduction

The thyroid gland is an essential endocrine gland that has a significant role in the body's growth, metabolism, and maturation.The thyroid gland helps to control various biological processes by continuously releasing several thyroid hormones into the bloodstream. The body releases extra hormones under certain circumstances, such as when it's cold, when it needs more energy, or when a woman is pregnant (1). The activity of the thyroid gland is controlled by the hypothalamic-pituitary-thyroid axis, which involves several hormones like thyrotropin releasing hormone (TRH), thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) (2). In particular, T4 plays a significant role in regulating thyroid function., the primary thyroid hormone, is a hormone released by the thyroid that is a biologically active hormone triiodothyronine (T3) in peripheral tissues (3).In recent years, Iraqi populations have experienced an increase in the prevalence of hyperthyroidism and hypothyroidism, two thyroid function diseases, particularly in Kirkuk Province. Healthy body growth depends on minerals including zinc, copper, and magnesium as well as vitamins D3 and B12. A few diseases, including thyroid issues, have been connected to variations in their levels (4). Hypothyroidism is the most common pathologic hormone deficiency. Hypothyroidism can be classified into various subtypes based on the time of onset (present from birth or developed later in life) and the severity of the underlying endocrine disorder "primary, secondary or central hypothyroidism". Additionally, It is a prevalent condition that has a higher incidence among women than men (5). To assess the prevalence of hypothyroidism in a community, serum thyrotropin is employed. Biochemically, to differentiate between overt and mild hypothyroidism, the serum-free thyroxine level must be either within or lower than the reference range. Iodine deficiency is still typical in cases where it is congenital (6). As opposed to congenital hypothyroidism, acquired hypothyroidism is brought on by autoimmune thyroiditis (Hashimoto's disease) (7).Low levels of circulating thyroid hormones and a decrease in thyroid hormone production are symptoms of hypothyroidism. Pituitary or thyroid gland failure was the cause of hyperthyroidism(8).Trace elements have a variety of effects on hormones, including binding to target tissues, activity, and secretion(9).The function of hormones, such as their synthesis, activity, and binding to target tissues, can be influenced by trace elements. Conversely, hormones can also impact the transport and excretion of trace minerals. This is because trace elements have the ability to act as activators or inhibitors in enzyme reactions, trace elements are necessary for a range of biological processes (10).Thyroid hormones are a vital factor in metabolism as they control the production of protons in tissues and the pace of metabolic reactions (11,12). A lack of thyroid hormone slows down a number of metabolic processes(13).As a catalyzer, zinc has a special role in the enzyme, helping to both regulate gene expression and convert thyroxin to its active form (thyronine)(14). Because it has an impact on growth and endocrine balance, zinc is crucial for the endocrine system. A lack of zinc in the body can reduce the release of thyroid hormone, which can change both the resting metabolic rate and the body's regular metabolism. According to certain studies, a zinc deficit is accompanied by an increasedproduction of the hepatic thyroxin-5-mono deiodinase enzyme, which catalyzes the activation of thyroid hormone(15,16).The thyroid gland uses copper, the third most frequent trace element in the human body, to help with hormone production, absorption, and metabolism. By inhibiting excessive T4 absorption in blood cells, copper controls calcium levels .Moreover, copper is required for phospholipid formation, which increases the production of thyroid hormones(17,18). Magnesium (Mg) is crucial in the development of thyroid illness. Mg appears to be involved in DNA replication, transcription, and repair as well as maintaining the structural stability of nucleic acids. Thus, any Mg deficit might cause DNA alterations that result in the growth of malignancies (19). Thyroid cancer and serum Mg levels are strongly connected, and Malignant tumors are known to exhibit higher levels of Mg compared to healthy tissues (20, 21). In contrast, thyroid cancer patients' serum Mg levels are lower than those of healthy individuals (22).Iron (Fe) is necessary for human health because it takes part in oxidation-reduction processes and facilitates the movement of oxygen throughout the body. Thyroid peroxidase(TPO), a heme-dependent protein, catalyzes the first two steps in producing thyroid hormone. Pregnancy, the immunological systems, and cognitive development are all negatively impacted by Fe deficiency (23). The production of thyroid hormones can be hampered and TPO activity reduced by severe Fe deprivation (24).In this study, serum samples were utilized to look into the correlation between a few trace elements (Fe, Mg, Cu and Zn) in individuals who have hyperthyroidism and hypothyroidism versus a control group. We were motivated to undertake this study due to the rise in thyroid patients. In Kirkuk, Iraq, there are contradictory findings regarding the contribution of trace elements to the emergence of thyroid disorders.

Material and methods

Time and Place: From October 2021 to June 2022, the present study was conducted at a private medical laboratory called Ibn Al-Nafees.

Study type: A study utilizing a cross-sectional design has been conducted.

Sample collections: 100 patients in all, including men and women, were selected; All patients reported thyroid dysfunction and had an average age of 38.14 ± 1.4 years, seven underwent thyroidectomy; and 31 were placed on a daily dose of 50 mg of thyroxin. For each patient, a unique questionnaire with all the necessary information was created. From each patient, five milliliters of venous blood had collected from them in a sterile manner. The blood sample was gently dispensed into a tube that had a jell-activator to aid in blood coagulation and the collection of clear sera (25). After 10 minutes of incubation. The tube was subjected to centrifugation at a speed of 3000 RPM for a duration of 5 minutes. When ready to use, Subsequently, the serum was carefully poured into a new, steriletest tube and kept at a temperature of 20°C for a maximum of 72 hours (26).The Mini-Vidas device was employed to measure the concentrations of TSH, T3, and T4, thyroid hormones using a fluorescent ELISA technique. In contrast, flame atomic absorption spectroscopy was used to measure trace elements Zn, Cu, Mg and Fe. All the tests were performed following the instructions provided with the targeted kit.

Thyroid hormones:

T3, T4, and TSH hormone levels were assessed utilizing Mini-Vidas equipment along with the specialized kits for each hormone. Each thyroid hormone was analyzed in accordance with the Biomerurix company's kit protocols, which were produced in France. A volume of 100 l of serum was used for the assessment of T3, The estimation of T4 involved the use of 200 l of serum.

Estimation trace elements in serum by FAAS:

1.Estimation of zinc and copper:

One milliliter of blood serum is taken and placed in a graduated cylinder and supplemented to (10 mL) with Distilled water, mixed and read directly into the machine (27).

2.Estimation of magnesium:

Take (50µl) of blood serum and put it in a graduated cylinder and supplement it with (5mL) of lanthanum Chloride, mix well and read directly (27).

3.Estimation of iron:

Five ml of blood serum is mixed with (25ml) HCL and kept in the incubator for half an hour, then (250m)l of tri-Chloro acetic acid (TCA) is added to the mixture and then (50ml) of the mixture is injected into the (AAS) machine (27).

Statistical analysis:

All of the gathered data was combined and arranged in a Microsoft Excel file. Statistical analysis was conducted to compare the parameters between the study and control groups, with a significance of $P < 0.05$ levels of significance using the chi-square and ANOVA tests.

Results:

Table 1 displays the results of the study, indicating a considerable rise in TSH hormone levels among patients with hypothyroidism and a noteworthy rise in T4 hormone levels among patients diagnosed with hyperthyroidism. The study also reveals a negligible increase in T3 hormone levels among patients with hyperthyroidism.

Table 1: T3, T4, and TSH hormones serum levels in both thyroid patient group and control group.

Thyroid state	T3 (nmol/L) Mean±SD	T4(nmol/L) Mean±SD	TSH(µIU/ml) Mean±SD
control	1.19±0.08	100.98±3.67	1.73±1.73
Hypothyroidism	1.14±0.19	74.64±0.45	21.30±2.11
Hyperthyroidism	1.34±0.015	162.97±16.97	0.13±0.005
p- value p ≤ 0.05	0.77	0.0018	0.0001

TSH, T4, and T3 levels in patients with hypothyroidism were as follows (21.30µIU/ml), (74.64) and (1.14) nmol/L, respectively. And in the patients with hyperthyroidism were follows (0.13 µIU/ml), (162.97 nmol/L), and (1.34 nmol/L) respectively, compared to the control group were followed (1.73 µIU/ml), (100.98 nmol/L), and (1.19 nmol/L) respectively.

Results have clearly shownlower concentrations of both zinc and magnesium in hypothyroidism and hyperthyroidism patients compared to healthy ones. whereas a notable decrease concentrations was seenin copper and iron in patients with hypothyroidism, and an increase in their concentrations in hyperthyroidism patients compared to the healthy ones, see Table 2.

Table 2: Trace elements concentrations in serum including copper, zinc, magnesium and iron.

Thyroid state	Copper (ppm) Mean±SD	Zinc(ppm) Mean±SD	Magnesium (ppm) Mean±SD	Iron (ppm) Mean±SD
control	1.32± 0.22	0.90 ± 0.09	20.5 ± 0.13	1.32 ± 0.29
Hypothyroidism	1.21± 0.24	0.74 ± 0.05	17.56 ± 0.41	1.11 ± 0.25
Hyperthyroidism	1.52 ± 0.25	0.85 ± 0.07	18.37 ± 0.66	1.71 ± 0.48
p- value p ≤ 0.05	0.048	0.007	0.042	0.041

The concentration of magnesium and zinc in hypothyroidism patients was recorded as (17.56 ppm) and (0.74 ppm) respectively, and in hyperthyroidism patients was (18.37 ppm) and (0.85 ppm) respectively, compared to the control group which had concentrations of (20.5 ppm) and (0.90 ppm) respectively. On the other hand, the concentration of copper and iron in hypothyroidism patients measured to be (1.21 ppm) and (1.11 ppm) respectively, and in hyperthyroidism patients was (1.71 ppm) and (1.52 ppm) respectively, compared to the control group which had concentrations of (1.32 ppm) and (1.32 ppm) respectively.

Discussion:

Prior to the last three or four decades, thyroid dysfunction was unheard of in Iraq, Since 1980, with the change in lifestyle, there has been an increase in awareness of most chronic illnesses (28). In addition, The present investigation has observed a prevalence of 14.28% for atypical thyroid activity which may have different underlying causes, which include the economic sanctions, Iraq war, political instability, events and crises that occurred after 2014 that caused the displacement of Iraqi residents from certain regions. The deteriorating infrastructure, loss of employment opportunities, and poverty have led to malnutrition and deficiencies in essential vitamins and trace elements, including but not limited to vitamin B12, D3, Zn, Se, and iodine. The lack of these nutrients and contribute to the development of hypovitaminosis (29) can ultimately affect the production of thyroid hormones (30).

The present study's findings agree with (Al-Abdulaziz, B. A., et al) (31), which revealed that the TSH hormone level of significantly increases inpatients withhypothyroidismalong with a low level of T4 and T3 hormones, while the TSH hormone level significantly decreases in patients with hyperthyroidism with an increase in the level of T4 hormone. Also a non-significant increase in thelevel of T3 hormone was recorded.The reason for the TSH hormone level increase in hypothyroidism with a decrease of T4 and T3 hormones level is due to that the hypothalamus secretes TRH, which in turn triggers the secretion of TSH from the pituitary gland. Increased concentrations of free T4 and T3 activate a feedback mechanism that suppresses the production of both TRH and TSH, thereby reducing the uptake of iodine and secretion of T3 and T4. Additionally, TSH production can also be hindered by other hormones, such as somatostatin, glucocorticoids, and dopamine (32). Where both (Shahid, M. A., *et al*)(33) and (Pirahanchi, Y., *et al*)(34) Shahid and Pirahanchi's study suggested that the reason TSH levels increase in primary hypothyroidism is due to the negative feedback inhibition loss, which is similar to the case of Hashimoto's thyroiditis. In cases of secondary hypothyroidism, such as those caused by a benign pituitary gland tumor, there is a decrease in TSH levels, as described by Shahid and Pirahanchi in their research. Thyroid hormone replacement therapy is part of the treatment for hypothyroidism(35,36,37). According to the negative feedback mechanism, TSH levels tend to decrease in primary hyperthyroidism, which is caused by a thyroid adenoma, due to the inhibitory effect of T3 and T4 on the anterior pituitary gland. On the other hand, In secondary hyperthyroidism, such as in the scenario of a tumor that produces TSH or TRH, both TSH and T3/T4 levels rise (38,39).

Table 2 was utilized to examine Fe, Mg, Cu, and Zn levels in the serum of patients with hypothyroidism and hyperthyroidism, and To investigate a possible connection between these trace elements and the functioning of the thyroid gland. In this study, The level of iron was found to be markedly higher in patients with hyperthyroidism than those with hypothyroidism or the control group, and this outcome corresponded with previous research (Moustafa, I. M., *et al*)(40), The results indicate that the range of iron concentration in the blood serum is elevated in cases of hyperthyroidism compared to the control group. Due to an increase in oxidation levels and radicals that result in cell wall destruction, hyperthyroid patients showed a decrease in the activity of glutathione peroxidase, glutathione andsuperoxide dismutase, with an increase in iron ion concentration in their blood. As a result, problems with the elements' equilibrium and problems with the disease's resultant metabolism will subsequently arise (41). The current study shows a significant decrease in Mg levels among patients with hyperthyroidism and hypothyroidism in comparison to the control group, and these findings are consistent with the results reported by Matha Srinivas et al (42), The present biochemical evidence suggests that maintaining thyroid function requires not only dietary iodine but also other elements such as Mg, Fe, Se, and others. The active process of iodine uptake is significantly influenced by the presence of Magnesium-ATP (43). Recently, an observational study showed an acquired Mg deficient state as the cause of a mitochondrial malfunction, which can account for the changes linked to thyroid disease (44). Although thyroid tissue appears to "just" absorb iodine (45),This mechanism is classified as active transport and thus necessitates energy.According to Tyler's study in 1968, the absorption of iodine is influenced by the existence of Mg-ATP (46). Following iodine absorption, thyroid peroxidase interacts with tyrosine residues located on the thyroglobulin molecule in order to create thyroid hormones (47). Research has shown that the activity of peroxidase, which is responsible for synthesizing thyroid hormones, can be stimulated by magnesium, thereby leading to an increase in thyroid hormone production, this action is mostly regulated by TSH (48).Zinc concentration was significantly decrease in hypothyroidism and hyperthyroidism compared to healthy subjects and these results agree with Arora, M., *et al* (49) and Khadem-Ansari, M. H., *et al* (50). Zinc plays a crucial role in protein synthesis and thyroid metabolism, and the decline in zinc levels in the blood serum of patients with hyperthyroidism leads to heightened activity of the enzyme deiodinase, which transforms (T4) to (T3), which increases the active thyroid hormone (T3), which affects thyrotropin-releasing hormone to stimulate (TSH) (51,52), while in hypothyroidism patients the decrease in Zn concentration leading to lower activity of (hepatic-1,5-deiodenase) by 67%, This implies that the conversion of T4 into the active T3 will be reduced, as a consequence, there will be a reduction in the concentration of both T3 and T4 hormones, as well as a decline in the concentration of the enzyme glutathione peroxidase (53). Low dietary intake of Zn can result in Zn insufficiency. Another study by Prasetyoand co-workers found that about 74% of adults in Indonesia consume little Zn (54).A study conducted in Pakistan discovered that individuals with hyperthyroidism had a lower average zinc level in their blood serum compared to healthy individuals in the control group, this implies that there may be a correlation between the levels of zinc and thyroid function (55). Another research study found that hyperthyroidism patients had higher levels of zinc in their blood serum than hypothyroidism patients and that administering zinc supplements can assist in preventing thyroid function problems (51).The current investigation also demonstrated a noteworthy reduction in copper levels among individuals with hypothyroidism and a significant increase in patients with hyperthyroidism compared to healthy people, These results were consistent with what was previously obtained by Moustafa, I. M., et al (40). The decrease in Cu concentration in hypothyroidism is leading to a reduction in Glutathione peroxidase concentration then a drop in super oxide dismutase concentration consequently. In this case, the risks of cell destruction will be increased, and the formation of free radicals (53). According to the results, hyperthyroidpatients have elevated plasma Cu concentrations compared to their control subjects. There are two potential explanations for this observation: the initial one being that a deficiency in zinc may enhance the absorption of copper from the intestine, as suggested by the literature (56). The other possible mechanism is that since ceruloplasmin transports about 90% of copper in the bloodstream, hyperthyroid patients have significantly higher ceruloplasmin levels (57) Thus, increasing Cu level in hyperthyroidism can be explained by increased ceruloplasmin and sluggish Cu elimination from the body.

Conclusions:

Hypothyroidism and hyperthyroidism are associated with changes in trace elements (Zn, Cu, Mg, and Fe); all studied elements decreased in hypothyroidism while (Fe and Cu) were elevated in hyperthyroidism, The findings of the study suggest that there are disparities in the concentrations of particular elements among individuals with thyroid disorders as compared to those who are healthy. However, further research with a larger sample size is needed to confirm and rely on these findings for regular examination in cases of thyroid dysfunction.

Conflicts of Interest

We, the authors, state that there is no conflict of interests.

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