Original paper

Assessment of serum essential elements, serum glucose and thyroid function tests in Iraqi hypothyroid patients have good and poor responses to levothyroxine therapy

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Abstract

Background: Hypothyroidism is a syndrome resulting from deficiency of thyroid hormones leading to generalized slowing of all metabolic processes. Many diseases like hypertension, ischaemic heart disease, adverse lipid profile, and Metabolic abnormalities developed in hypothyroid patients. Trace elements play essential roles in the human body as cofactors of some enzymes and take part in the synthesis of many hormones where normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones.

Aim: The present study was designed to evaluate fasting serum Fe, Cu, and Zn concentrations, fasting serum glucose concentration and thyroid function tests in hypothyroid patients having good and poor response to LT therapy and to study correlations between these parameters.

Materials and methods: The present study was a single center, cross sectional study conducted in alshefa General hospital, basra, Iraq. During the period from June,2013 to Mar, 2014. 62 subjects aged 20 to 55 years were included. The patient groups consisted of 41 hypothyroid patients (38 females and 3 males) on LT therapy for at least 6 months duration. 21 Healthy age and gender matched group with thyroid function within normal limit were selected as control group. Patients were screened for FT3, FT4 and TSH, those with high TSH level above normal limit despite high dose of thyroxine considered as poor response to treatment (PRLT) while those patient with TSH level within normal range considered as good response to treatment (GRLT). After 12 hrs fasting, blood samples (5 ml) were obtained to evaluate serum TSH, T3, T4, glucose and trace elements in all studies groups.

Results and discussion: In (GRLT)group 21 patients had thyroid function tests (TSH, FT4 and FT3) within normal range and there were no significant differences when compared with control while in (PRLT) group, FT4 and FT3 significantly decreased when compared with control and GRLT groups while serum TSH levels in all 20 hypothyroid patients were significantly high. Serum Zn, Cu and Fe level of PRLT patients are significantly lower than its level in control and (GRLP) groups, In (GRLP) there were no significant differences when we compared trace element concentrations with control. Regarding FSG no significant differences were reported in all studied groups. There are significant correlation between thyroid function tests and trace element concentrations. Trace elements influence thyroid hormones at levels of action, including hormone secretion and activity and binding to target tissue. On the other hand, thyroid hormones itself influence trace metals metabolism at several levels of action, including excretion and transport of trace metals. All these factors collectively lead to significant reduction in trace elements concentrations in hypothyroid patients compared to normal control .

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Furthermore many reports demonstrate that effect of overt hypothyroidism on glucose metabolism is still a subject of debate this came in agreement with our study. **Conclusions:** There are significant decrease in serum Zn, Cu and iron in hypothyroid patients have poor response to LT therapy compared to normal subject. There is no significant change in serum glucose concentrations in all tested groups. Our finding revealed significant correlation between thyroid function tests and serum trace elements.
Key words: essential elements, serum glucose, hypothyroidism, levothyroxine

Introduction

Hypothyroidism is a syndrome resulting from deficiency of thyroid hormones namely thyroxine (T4) and tri-iodothyronine (T3), leading to generalized slowing of all metabolic processes, it was divided in to overt and subclinical hypothyroidism in both serum thyroid-stimulating hormone (TSH) levels outside the reference range [1,2]. The prevalence of hypothyroidism varied from 2-5%, increasing to 15% in elderly over 75 years, with a higher incidence in females [3]. Many processes in the body like in growth, development, and metabolism in humans are under the direct or indirect control of the thyroid hormones. Full activity of the thyroid hormones requires the deiodination of T4 toT3, which capable of modulating expression of control regions of target genes[4]. Actually many diseases like hypertension, ischemic heart disease, adverse lipid profile, endothelial dysfunction and Metabolic abnormalities have been suggested to be the major consequences of hypothyroidism, [5-7]. Meanwhile hypothyroidism in infants and children results in growth and mental development retardation [8]. So successful treatment are required to normalize thyroid hormone levels in peripheral tissues this accomplish by using Levothyroxine replacement therapy for reversing such direct or indirect problems [9]. There were cross connection between thyroid hormones, insulin and trace elements poorly discuses that may lead to unexpected or predictable complications [10,7]. It is well known that trace elements play essential roles in the human body as cofactors of some enzymes and take part in the synthesis of many hormones where abnormal level may be the end results of many disease or the causes of some diseases thus precise determination and monitoring of trace elements has clinically become an important key marker for diagnosing wide diseases [11]. Normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones[12] In other hand hypothyroidism may affect trace element levels where many studies have been reported that the disorder of thyroid hormones can influence the homeostasis of trace metals in the body, such as Ca, Mg, Mn, Cu, and Zn [13]. prevalence of anemia in patients with hypothyroidism has been shown to be high (20-60%) [14,15].

Thyroid hormones are important mediators of glucose homeostasis, it can stimulate the expression and activation of large numbers of proteins that are candidates for regulating insulin sensitivity via sympathetic projections to the liver [16] this fact could explain the relationship between thyroxine and serum glucose level. For these huge effects, T4 therapy, using synthetic levothyroxine (LT), is the standard of care for thyroid hormone therapy in patients with hypothyroidism [17]. Moreover there were variations in hypothyroid patient's response to T4 therapy so measurement of serum thyroid stimulating hormone is the cornerstone of monitoring LT replacement, the exception being people with pituitary disease. After starting LT, thyroid stimulating hormone and free thyroxin levels should be measured at8 to 12 weeks and adjustments

made to the dose accordingly [18]. Many patients still having high TSH level despite adequate doses of T4 , Patient non-compliance with LT therapy can sometimes be a problem and less than 10% of patients poorly responded to LT, with no clear reasons [19]. In view of the role of trace element as a constituent of T3 nuclear receptors and may involve in T4 T3 conversion [20], Therefore, in order to illustrate the influence of LT therapy on the homeostasis of trace elements, serum glucose level, the objective of current study we compare fasting serum Fe, Cu, and Zn concentrations, fasting serum glucose concentration and thyroid function tests in hypothyroid patients having good and poor response to LT therapy with age and gender matched individuals as control group, searching for reasons or correlations that explain such controversy in response.

Materials and methods

The present study was a single center, cross sectional study conducted in alshefa Ceneral hospital, basra, Iraq. During the period from June,2013 to Mar, 2014. 62 subjects aged 20 to 55 years were included. The patient groups consisted of 41 hypothyroid patients (38 females and 3 males) on LT therapy for at least 6 months duration. 21 Healthy age and gender matched group with thyroid function within normal limit were selected as control group. The study protocol was approved by the local clinical research ethics committee. Patients were given detailed information about the aims of the study and written consent forms were obtained. Patients with hypothyroidism on LT therapy included in the study. Patients with interfering illnesses or who were taking drugs that could alter thyroxine absorption or metabolism were excluded. Patients were screened for FT3, FT4 and TSH. Based on these values, patients were either classified as cases of good response to LT therapy(TSH within normal range)(n=23)or as poor response to LT therapy and still having hypothyroidism(high TSH value)(n=18) according to Vaidya B and Pearce SH (2008) classification [19]. After 12 hrs fasting, blood samples (5 ml) were obtained from each patients and controls. Fasting venous blood specimens collected in the morning, specimens were placed on polyethylene tube; blood samples are left to clot and centrifuged at 10000 rpm for 20 minutes. The separated serum was divided into several aliquots for analysis and estimation of fT3, fT4 and TSH .The remaining serum samples were kept frozen for later analysis for the estimation of Zn, Cu and Fe.

Biochemical methods

Determination of thyroid hormones

Serum fT3, fT4 and TSH concentrations were by electrochemiluminescence immunoassay (ECLIA) technique intended for use on the Elecsys reagent kits supplied by Roche Diagnostics GmbH (Mannheim, Germany) and run on cobase 601 immunoassay analyzer from Roche Diagnostics Ltd, Switzerland. The reference range for serum fT3 (2.0-4.4 pg/mL), fT4 (0.93-1.7 ng/dL) and TSH (0.27-4.20 mIU/mL). fasting Serum glucose values were obtained through an enzymatic process Axthat uses the enzymes hexokinase and glucose-6-phosphate dehydrogenase, using test strip of the Accu-Chek® glucose meter

Determination of trace elements levels (Zn, Cu and Fe)

Concentrations of Zn, Cu and Fe were first must be released from the protein matrix by wet digestion method [21] and determined by atomic absorption spectrophotometer using a Buck Model 211-VGP spectrophotometer, with a detection limit of 0.005 ppm for Zn and Cu, and 0.05 ppm for Fe. The flame conditions were fixed as recommended by the instrument manufacturer for Zn, Cu and Fe, (wavelengths 214, 324, 247 nm, respective-ely). The band pass was 0.7 nm for Zn, Cu while 0.2 for Fe and the

measuring time was 3 second. Standard solutions (1000 ppm STD supplied by Buck company) of those elements were first aspirated to calibrate the atomic absorption spectrophotometer (AAS) before the aspiration of the samples. From the prepared standard curves the concentration of each element was calculated using the following formula (22): (Sample concentration= Read concentration × dilution factor) trace element concentrations were presents as (µg/dl).

Statistical analysis

Values were expressed as mean ± S.D; the values were statistically evaluated using unpaired Student's t-test and one way analysis of variance (ANOVA), supported by Bonferroni's post hoc analysis. Values with $P < 0.05$ were considered significantly different. Analysis was performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

Results

Most study subjects (92%) were women, 41 hypothyroid patients on LT therapy and 21 healthy subjects serve as control group. In the group with good response to levothyroxine therapy (GRLT) 21 patients had thyroid function tests (TSH, FT4 and FT3) within normal range and there were no significant differences when compared with control while in group with poor response to levothyroxine therapy (PRLT), FT4 and FT3 significantly decreased when compared with control and GRLT groups while serum TSH levels in all 20 hypothyroid patients were significantly higher than the control group. Regarding T3/T4 ratio there were no significant differences between all studied groups. All these data clearly summarized in figure (1).

The levels of serum trace elements (Zn, Cu and Fe) in both groups who had good or poor response to treatment as comparison with control group and each other are listed in Figure (2). The results of serum trace elements expressed as mean±standard deviation. Serum Zn, Cu and Fe level of PRLT patients are significantly lower ($p<0.05$) than the level in Control and (GRLP) groups, whereas, there were no significant difference ($p>0.05$) when we compared trace element concentrations between (GRLP) group and control.

In this study, we have analyzed fasting serum glucose (FSG) concentrations among hypothyroid treated groups and control. As shown in figure (3) we've seen FSG concentration decrease in PRLT group but not significantly when compared with control and GRLP groups.

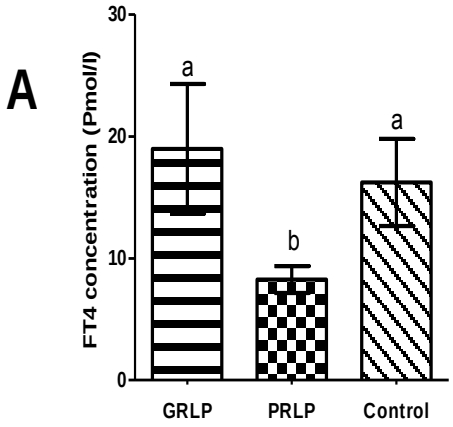
With such a high prevalence of both thyroid dysfunction and metabolic syndrome with trace elements deficiency we might suspect there are connections between these parameters. Accordingly In the present study, we have analyzed the correlation between thyroid hormones, fasting Serum glucose levels and trace elements among hypothyroid patients and control groups. Table(1) showed there were significant associations between FT4 and serum trace elements (Cu and Fe) concentrations in GRLT group while in PRLT the correlation observed with serum Cu and Zn. The same finding observed when we make correlation between FT3 and trace elements in both GRLT and PRLT groups. Moreover there were significant negative correlations between TSH and serum trace elements in GRLT while in PRLT the correlation observed with serum glucose and iron.

Discussion

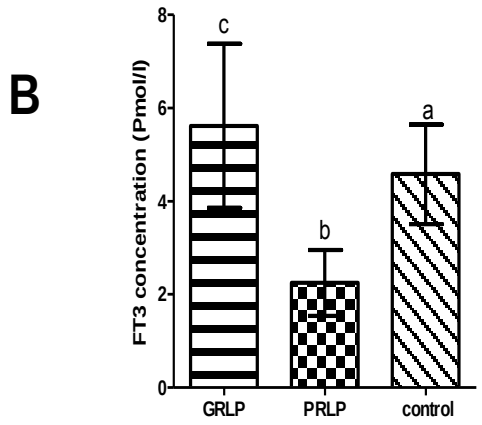
The maintenance of optimal health requires an adequate supply of carbohydrates, proteins lipids, macronutrients, micronutrients, and trace elements. [23]. Although there are some previous studies about the serum concentrations of thyroid hormone and trace elements in hypothyroidism , there is

little information about the effects and role of such trace element in hypothyroid patients have poor response to LT therapy. Trace elements play an essential role in large number of biological processes through their action as activators or inhibitors of enzymatic reactions, by influencing the permeability of cell membranes, or by other mechanisms.

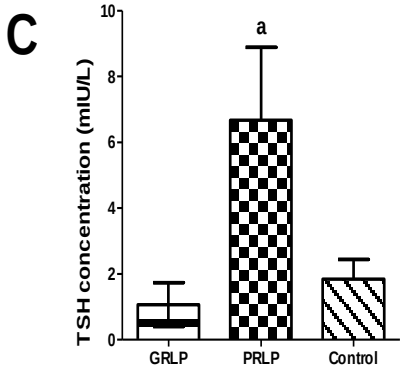
Figure(1) thyroid function tests in both GRLT and PR LT hypothyroid and control groups



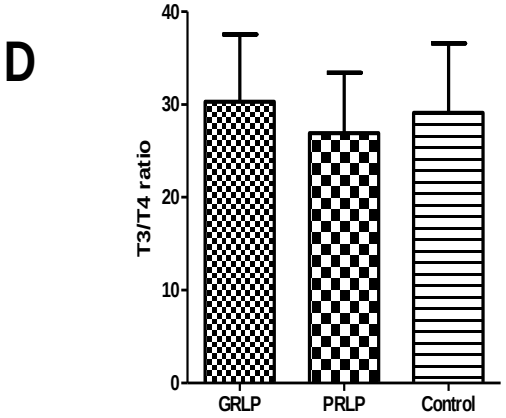
A)Serum free thyroxine concentrations. values were presented as mean±S.D; values with non-identical letters (a,b,c) represent significant differences



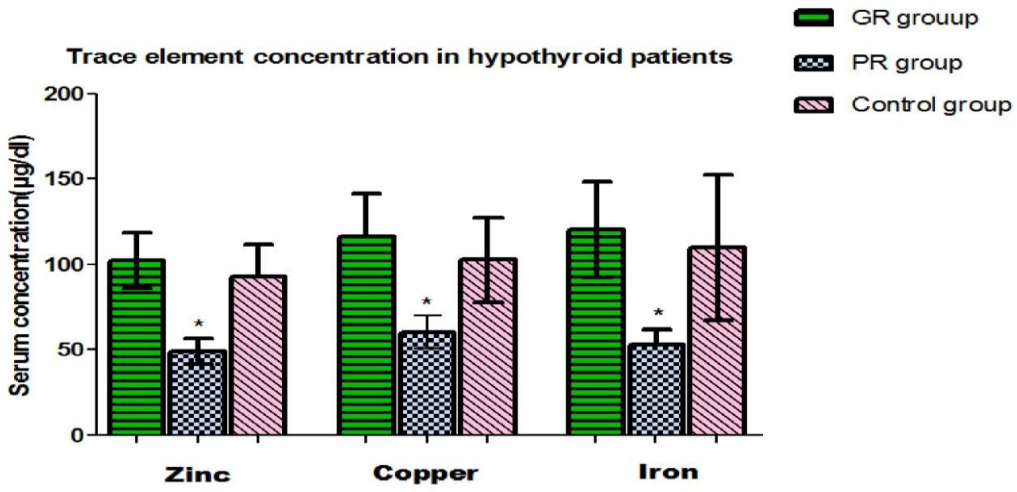
B)Serum free T3 concentrations values were presented as mean±S.D; values with non-identical letters (a,b,c) represent significant differences



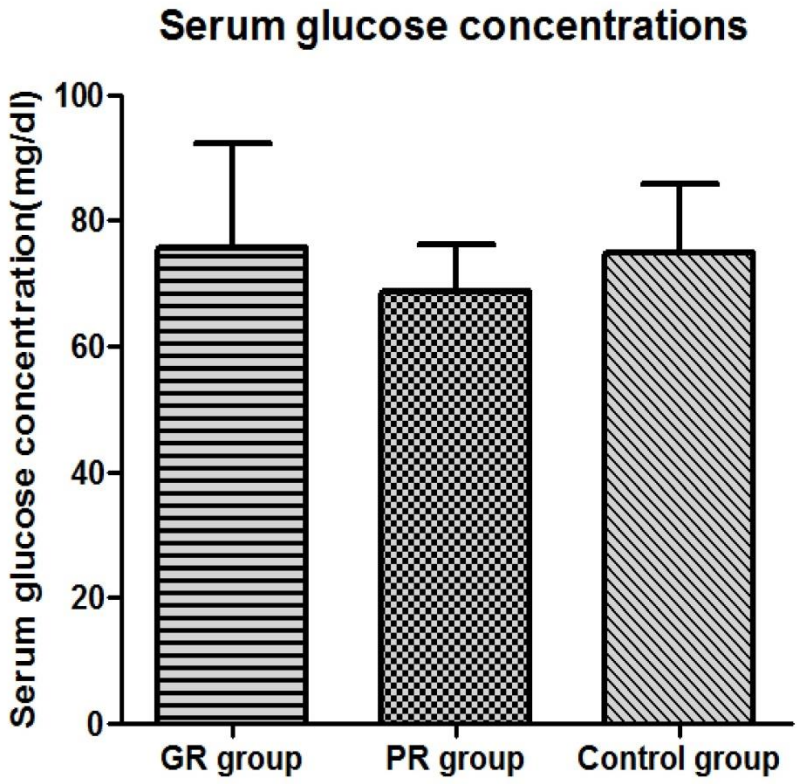
C)Serum TSH concentrationS values were presented as mean±S.D; values with non-identical letters (a,b,c) represent significant differences



D)Serum T3/T4 percentage. values were presented as mean±S.D; No significant differences



Figure(2) The concentration of Zn, Cu and Fe in Iraqi hypothyroid patients treated with levothyroxine in both good and poor responce treated groups as compared with control.values are presented as mean±S.D; * significantly different compared to control (P<0.05); no significant differences among treated groups (P>0.05).



Figure(3) The concentration of glucose in Iraqi hypothyroid patients treated with levothyroxine in both good and poor responce treated groups as compared with control.values are presented as mean±S.D; no significant differences among treated groups (P>0.05).

Table 1. Correlation analysis among different parameters of control, GRLT and PRLT groups in the studied subjects**.** Data expressed as mean ± SD. * = significantly different (P<0.05): ** = significantly different(P<0.0001)

Parameter	Group	Pearson Correlation Coefficient (P Value)			
		Serum Glucose	Serum Iron	Serum Copper	Serum Zinc
TSH	GRLP	0.3917 (0.1199)	-0.6998 (0.0018)*	-0.7496 (0.0005)*	-0.5493 (0.0224)*
	PRLP	0.6417 (0.0055)*	-0.4839 (0.0490)*	-0.2167 (0.4034)	-0.2207 (0.3946)
	Control	0.3291 (0.1970)	-0.4561 (0.0657)	-0.2531 (0.3269)	0.2423 (0.3487)
FT4	GRLP	-0.4666 (0.0590)	0.8722 (<0.0001)**	0.8555 (<0.0001)**	0.6376 (0.0059)
	PRLP	0.2897 (0.2593)	0.1647 (0.5275)	0.8516 (<0.0001)**	0.9060 (<0.0001)**
	Control	0.2850 (0.2674)	-0.3493 (0.1692)	-0.1334 (0.6096)	0.0565 (0.8293)
FT3	GRLP	-0.4702 (0.0568)	0.5811 (0.0144)*	0.5037 (0.0392)*	0.2243 (0.3868)
	PRLP	0.3836 (0.1284)	0.3185 (0.2127)	0.6646 (0.0036)*	0.6248 (0.0073)*
	Control	0.1573 (0.5464)	0.0306 (0.9070)	0.2509 (0.3312)	0.2338 (0.3664)

Trace elements influence thyroid hormones at levels of action, including hormone secretion and activity and binding to target tissue. In other hand, thyroid hormones itself influence trace metals metabolism at several levels of action, including excretion and transport of trace metals. [24,25] In the present study serum concentration of trace elements (Zn, Cu and Fe) decrease significantly in PRLT group as compared to control and GRLT groups, this could be an explanation describing a persistent high TSH level in PRLT group despite taking a high dose of LT. Such patients may require supportive therapy with trace element preparations in order to obtain desired response to LT therapy. Our finding came in agreement with many studies that conclude normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of thyroid hormones, for example selenium is required for conversion of T4 to T3 by deiodinase enzyme [26]. Furthermore copper deficiency enhances the effect of hypothyroidism and there are significant positive correlation between the serum concentration of copper and T3 [27,28]. Meanwhile, Cu deficiencies may be a result of thyroxine hormone insufficiency and the fortification of T4 may help to heal serum Cu disorders [29]. Zinc play a crucial role in thyroid function it has been found that Zinc supplementation appeared to have a favorable effect on thyroid hormone levels, particularly total T3, and resting metabolic rate, actually Zn is a constituent of T3 nuclear receptors [30]. In addition there was a significant correlation of serum zinc levels with thyroid volume in nodular goitre patients, with thyroid autoantibodies in AITD and with free T3 in patients with normal thyroid. [31]. These results came in tune with our study were significant positive correlation had been reported between Zn, Cu and iron with both T3 and T4. Moreover, Thyroid hormones influences zinc metabolism. In a study carried out in nephrectomized rats, it was observed that reduced thyroid function was strongly related to low serum zinc level [32].Prasad et al.(1999) reported that thyroid hormones not only affected by zinc deficiency, it may modulate zinc transport activity in rat renal and intestinal brush-border membrane affecting zinc level [33].One possible explanation for decrease serum zinc concentration, that gastrointestinal absorption of zinc is severely impaired in hypothyroidism subjects. An alternative explanation would be a change in zinc distribution; the low zinc level may reflect sequestration of zinc by the liver or other tissues [34,35]. In fact the role of iron, zinc and copper t in the thyroid are less well defined but sub-or super optimal dietary intakes of all these elements can adversely affect thyroid hormone metabolism [36]. Several literatures demonstrated low serum iron concentration in hypothyroid patients

similar to our finding. Studies in animals and humans have shown that iron deficiency with or without anemia impairs thyroid hormone metabolism meanwhile nutritional iron deficiency has been shown to significantly lower the circulating levels of both thyroxine and triiodothyronine in rats [37]- and reduce conversion of T4 to T3.[38]. Other study indicates that the degree of iron deficiency may affect thyroid hormone status in iron-deficient adolescent girls and The mean serum iron level was significantly lower than those of healthy individuals [39]. Conversely decrease iron level may adversely affect thyroid hormones concentration where secondary and subclinical hypothyroidism were found in iron deficiency anemia and hormonal changes returned to normal values with iron supplementation. One possible explanation that iron deficiency can affect the thyroid hormone metabolism and peroxidase enzyme which catalyzed initial steps of thyroid hormone synthesis that dependent on the iron. Other possible explanation in studies carried out in rats have indicated that iron is required during conversion of T4 to T3 [37]. Moreover, When iron and thyroxine were mixed together in vitro, a poorly soluble purple complex appeared that indicated the binding of iron to thyroxine and this interaction should be considered when these drugs administered together [40].

The present study observed no significant difference when we compared serum glucose concentrations in GRLT and PRLT groups with control subject. In contrast to this idea overt hypothyroidism showed increase levels of A1C and glycated albumin also abnormal glucose metabolism where thyroid hormone replacement is associated with a decrease in A1C level,. [41], however such decrement could be due to increased erythropoiesis rather than by changes in glucose level. Many studies revealed that T3 and insulin both stimulate the expression of hexokinases and glycogen synthase which are respectively responsible for uptake and disposal of glucose via formation of glucose-6 phosphate and glucose-1 phosphate . At a molecular level, microarray studies performed on mice liver have demonstrated that most of the enzymes involved in gluconeogenesis are positively regulated by thyroid hormone [42]. Other reports demonstrate that effect of overt hypothyroidism on glucose metabolism is still a subject of debate [43] this came in agreement with our study. The high TSH values and low FT4 in the present study in PRLT group give an idea about positive feedback mechanism on pituitary gland while in GRLT group TSH values normalize and return to reference range with normal FT4 level. This hypothalamus pituitary thyroid glands axis maintains normal thyroid secretion when exogenous thyroid hormones were administered. [44]

Conclusions

There are significant decrease in serum Zn,Cu and iron in hypothyroid patients have poor response to LT therapy compared to normal subject and hypothyroid patients having good response to LT therapy. There is no significant change in serum glucose concentrations in all tested groups. Our finding revealed significant correlation between thyroid function tests and serum trace elements.The findings underline the importance of trace element replacement in un controlled hypothyroidism .

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