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Evaluation of the role of insulin-like growth factor-1 and some minerals in treatment of hypothyroid rats: Hormonal study

Doua'a Sahi Hasoon

Radiological Technique Department, Al-Mustaqbal University College, Babylon, Iraq
Email: douaa.sahi@mustaqbal-college.edu.iq

Maysaa Adil Hadi

Department of Biology, College of Sciences, University of Babylon, Babylon, Iraq
Email: sci.maysaa.aadil@uobabylo.edu.iq

Tabrek Falah Naji

Radiological Technique Department, Al-Mustaqbal University College, Babylon, Iraq

Ali Mohammad Bahrami

Faculty of Veterinary Medicine, Ilam University

Abstract---Untreated hypothyroidism can contribute to hypertension, dyslipidemia, infertility, cognitive impairment, and neuromuscular dysfunction. The management of hypothyroidism focuses on ensuring that patients receive appropriate thyroid hormone replacement therapy and monitoring their response. Hormone and supplement therapy should have beneficial effects. The present study was designed to evaluate IGF-1 and some supplements in thyroid-stimulating hormone (TSH) and thyroid hormones (T3 and T4) concentrations in response to treatment with T4, IGF-1, Zn, Se, and Vit.B12 in experimentally induced hypothyroid rats. Forth-week-old male Wistar albino rats (weight 180–250 g). Rats were given methimazole to induce hypothyroid and were randomly divided into seven groups including those treated with levothyroxine T4, IGF1, T4 + IGF1, T4 + Zinc, T4 +Selenium, T4 +B12, and T4+Zinc+ Selenium+ B12. The body weight was evaluated for each group before and after the treatment. The concentrations of TSH and thyroid hormones were measured using the ELISA technique. The data showed that the animal model of hypothyroidism had increased body weight in comparison to the control groups ($P \leq 0.05$). The body weight of rats in treatment groups with IGF-1 was significantly increased from (221 ±

16.8) g to (276.2 ± 25.9) g. A similar pattern for T4 and Zn alone was exhibited but the treatment with T4, Se, and B12 did not change body weight significantly ($P>0.05$). In addition, the animal model of hypothyroidism had a significant increase ($P\leq 0.05$) in the TSH concentration compared to control groups. In the animal treated with the IGF1 +T4 group significant decrease ($P\leq 0.05$) in TSH concentration and increased T3 and T4 were confirmed. However, there was no significant decrease in TSH concentration in hypothyroidism treated with T4 + Se and B12 group. The results of this study showed that the treatment used could cause improvement in the body weight changes and TSH, T3, and T4 hormones concentrations in hypothyroidism-treated groups with fewer side effects and has the potency to be used in the treatment of hypothyroidism.

Keywords---TSH, T3, T4 Hormones, IGF-1.

Introduction

The human body is made up of glands that secrete hormones. One of these glands is the thyroid gland or butterfly gland, which is located just below the larynx on both sides and in front of the trachea. This gland normally weighs 15 to 20 grams in adults and is composed of several follicles (Mohebbati and Shaha, 2012). The most important function of this gland is the secretion of thyroxine (T4) and triiodothyronine (T3), both of which affect the body's metabolism and have a profound effect on increasing the basal metabolism of all cells in the body, protein synthesis, regulating bone growth, and the maturation of nerve cells. Complete lack of thyroid secretion usually reduces basal metabolic rate to 40 to 50 percent of normal (Rousset *et al.*, 2015). The secretion of these hormones is controlled by a thyroid-stimulating hormone (TSH), which is secreted from the anterior pituitary gland (Nilsson and Fagman, 2017).

Thyroid hormones (TH) play critical roles in differentiation, growth, and metabolism. Indeed, these hormones are required for the normal function of nearly all tissues, with major effects on oxygen consumption and metabolic rate. Disorders of the thyroid gland are among the most common endocrine maladies. Furthermore, endemic cretinism due to iodine deficiency remains a public health problem in developing countries at the advent of the third millennium. Thus, the study of TH action has important biological and medical implications (Yen, 2001). The thyroid hormone regulates a wide range of genes after its activation from the prohormone T4, to the active form, T3. The signaling pathway is complex and highly regulated due to the expression of cell and tissue-specific thyroid hormone transporters, multiple thyroid hormone receptor (TR) isoforms, and interactions with corepressors and coactivators. Furthermore, thyroid signals are involved in cross-talk with a range of other signaling pathways (Brent, 2012). This study aimed to evaluate the role of Insulin-Like Growth Factor-1 and some supplements in the treatment of hypothyroid rats at hormonal levels and body weight.

Materials and Methods

Methimazole solution

The Methimazole was given as a 0.02% solution in drinking water for three weeks to induce a significant decline in T4 and T3 levels (Cakic-Milosevic *et al.*, 2004).

Levothyroxine solution

For most patients, therapy can be initiated with a full replacement dosage (1.6 g/kg body weight), which is usually 75 to 100 g/day for women and 100 to 150 g/d for men (Mandel *et al.*, 1993).

IGF-1 solution

The dose of IGF-I solution (2 µg. 100 g⁻¹. Day⁻¹) was used (María García, 2005).

Zinc and selenium solution

The solution of 30 mg Zn as zinc-gluconate and 200 mg Se as high-selenium yeast were used (Salma 2015).

B12 solution

The dose of B12 solution was 500 g/day (Kennedy, 2016).

Experimental Animals

Eight-week-old male Wistar albino rats (weight 180–250 g) purchased from Ilam Lab Animals (Ilam, Iran) were housed individually in cages. The rats were housed for a week before experiments in the cages where they were to be tested. The temperature was maintained at 22.2-23.3°C and relative humidity was controlled at 55%. The lights were on from 7:00 AM - 7:00 PM and they were off from 7:00 PM -7:00 AM. The rats were handled daily for treatment and the rats in group control were handled daily to reduce the effects of stress. The procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Ilam University . Normal rats were randomly divided into nine groups (each n=8); the negative control group, hypothyroid (positive control group), and treatment groups:

- Normal Control group: Included healthy intact animals which were given distilled water orally .
- Hypothyroid control group: Included animals that had been given Methimazole only orally.
- Treatment groups: Included 56 rats, they were treated with potent antithyroid drug methimazole (MMI) solution to induce a significant decline in T4 and T3 levels, and then, after the induction of hypothyroidism, they were treated orally with different minerals and Vit.B12. These hypothyroid treated groups were subdivided into seven subgroups (n=8):
 - The hypothyroid group was treated with levothyroxine (T4).

- The hypothyroid group was treated with IGF1.
- The hypothyroid group was treated with T4 + IGF1.
- The hypothyroid group was treated with T4 + Zinc.
- The hypothyroid group was treated with T4 +Selenium.
- The hypothyroid group was treated with T4 + Vit.B12.
- The hypothyroid group was treated with T4+Zinc+ Selenium+ Vit.B12.

Throughout the study period, the body weight of animals was measured and after sacrificing the animals, the concentration of TSH, T4, and T3 hormones were measured.

Blood sampling

At the end of the experimental period, the rat is deeply anesthetized. To ensure prolonged anesthesia, a cotton piece soaked in chloroform anesthetic was placed in a desiccator during the procedure. Blood was obtained by cardiac puncture and the blood was collected by a sterile needle. The blood sample is taken from the heart, from the ventricle slowly to avoid collapsing of the heart. After collection, the clotted blood was centrifuged and serum was collected to measure TSH, T4 & T3 hormones concentrations by ELISA technique using a commercial kit.

The Quantitative Determination of Thyrotropin, Total Thyroxine, and Total Triiodothyronine Levels in rat Serum

The quantitative determination of TSH concentration in rat serum by an ELISA technique was done using Calbiotech kit (USA) while T4 and T3 concentrations were done by Pishgaman Sanjesh kit (Iran) following the manufacturer's instructions.

Statistical Analysis

Statistical analysis using SPSS software (Version 21) based on ANOVA analysis for effects of each treatment in TSH, T3, and T4. Also, a t-test was performed for examination of the effects of each treatment on total body weight. The means differences were considered significant at ($P \leq 0.05$). Continuous variables were presented as (Means \pm SD).

Results

Body Weight

Results of this study showed some changes in body weight in experimental animals. There was a significant increase ($P \leq 0.05$) in the weight of hypothyroid animals from (254.4 \pm 24.1) gm to (297.5 \pm 45.3) gm, and hypothyroid treated with T4+ Zn+ Se+ and Vit.B12 group from (212 \pm 7.5) gm to (271.5 \pm 30.5) gm compared to normal control from (232 \pm 13.9) gm to (255 \pm 19.4) gm. Whereas T4 treated group increased from (227.6 \pm 21.6) gm to (268.6 \pm 31.7) gm, IGF1 treated group from (221 \pm 16.8) gm to (276.2 \pm 25.9) gm, T4 +IGF1 treated group (245.8 \pm 13) mg to (288.2 \pm 13) and T4+ Vit.B12 treated group from (229.4 \pm 18.5) gm to

(248.8 ± 11.26) gm caused nonsignificant increase compared with both normal and hypothyroidism groups.

Also, treatment with T4+Zn which increased from (184.2 ± 34.9) gm to (260.4 ± 32.9) gm caused a significant increase compared with both normal control and hypothyroidism groups while treatment with T4+Se increased from (226.4± 12.9) gm to (240.4 ± 18.4) caused nonsignificant differences compared to normal control and a significant decrease compared with hypothyroidism group. Furthermore, groups which treated with T4, IGF1, T4+IGF1, T4+Se, T4+ Vit.B12 could reduce negative effects on body weight caused by hypothyroidism that produced a significant increase in body weight compared to the normal group as shown in Table -1.

Table 1
Body weight changes in control and treated groups (means ± SD)

Groups	Body weight (g) (mean ± S.D)		Weight Change (g)
	Initial Body Weight	Final Body Weight	
Control	232 ± 13.9	255 ± 19.4	23 a
Hypothyroid	254.4 ± 24.1	297.5 ± 45.3	57.72 b
Hypothyroid+T4	227.6 ± 21.6	268.6 ± 31.7	41 ab
Hypothyroid+IGF1	221 ± 16.8	276.2 ± 25.9	55.2 ab
Hypothyroid+T4+ IGF1	245.8± 13.	288.2 ± 13	42.4 ab
Hypothyroid+T4+Zn	184.2 ± 34.9	260.4 ± 32.9	76.2 c
Hypothyroid+T4+ Se	226.4± 12.9	240.4 ± 18.4	20.8 ad
Hypothyroid+T4+ B12	229.4±18.5	248.8 ± 11.26	24.22 ab
Hypothyroid+T4+Zn+ Se+ B12	212 ± 7.5	271.5 ± 30.5	59.5 bc

Different letters refer to a significant difference between groups.

Similar letters refer to the non-significant difference between groups.

Hormonal assay

In table -2, the results showed a significant increase ($p < 0.05$) in TSH level in hypothyroid rats (3.57 ± 1.39 mlU/L) as compared to normal control (0.50 ± 0.10 mlU/L) while TSH level had a non-significant decrease in hypothyroidism treated with T4 group (1.28 ± 0.81 mlU/L), hypothyroid treated with T4 and IGF1 (0.60 ± 0.11), and hypothyroid treated with T4 and Se (0.61 ± 0.28 mlU/L) as compared to the normal group but considered as a significant decrease as compared to the hypothyroid group. Also, a significant decrease in TSH level in hypothyroid treated with IGF1 group (1.73 ± 1.11 mlU/L) as compared to a hypothyroid group, while a significant difference in TSH level (1.87 ± 0.01 mlU/L) in hypothyroid

treated with T4&Zn group, hypothyroid treated with T4& Vit.B12 group (1.72 ± 0.92 mIU/L), and hypothyroid treated with T4, Zn, Se, and Vit.B12 group (1.67 ± 0.71 mIU/L) as compared to both hypothyroid and normal groups.

In addition, in the same table (4-2) the results recorded a significant decrease in T3 level in hypothyroid (0.92 ± 0.03 ng/ml) and hypothyroid treated with the T4 group (0.94 ± 0.06 ng/ml) as compared to the normal control group (1.11 ± 0.09 ng/ml). The nonsignificant difference in T3 level in hypothyroid treated with IGF1 group (1.02 ± 0.13 ng/ml) as compared with hypothyroidism and normal groups, while a nonsignificant increase in T3 level in hypothyroidism treated with both T4& IGF1 group (1.10 ± 0.21 ng/ml) and hypothyroid treated with T4& Se (1.08 ± 0.15 ng/ml) as compared with normal control and hypothyroid groups. Also, nonsignificant differences in T3 level in hypothyroid treated with T4 & Zn group (1.10 ± 0.14 ng/ml) and T4&IGF1 group (1.10 ± 0.21 ng/ml) as compared with the normal control group but considered as a significant increase as compared to the hypothyroid group.

Furthermore, a significant decrease in T3 level in hypothyroid treated with T4& Vit.B12 group (0.88 ± 0.15 ng/ml) and hypothyroid treated with T4, Zn, Se, and Vit.B12 (0.9 ± 0.08 ng/ml) as compared with normal control and hypothyroid groups. Table (2) also showed a significant decrease in T4 level in the hypothyroid group (3.02 ± 1.23 µg/dl) as compared with the normal control group (4.18 ± 1.05 µg/dl). While nonsignificant differences in T4 level in the hypothyroid rats treated with the T4 group (4.12 ± 0.57 µg/dl), hypothyroid treated with IGF1 group (3.1 ± 1.87 µg/dl) and hypothyroid treated with both T4&IGF1 group (3.54 ± 1.36 µg/dl) as compared with normal control and hypothyroid. Also, a significant increase in T4 level in hypothyroid treated with the T4&Zn group (5.18 ± 1.14 µg/dl) and hypothyroid treated with the T4&Se group (4.92 ± 0.53 µg/dl) as compared with the hypothyroid group. Also, nonsignificant decrease in T4 level in hypothyroidism treated with T4& Vit.B12 group (4.06 ± 0.52 µg/dl) as compared with normal control and hypothyroid groups while a significant increase in T4 level in hypothyroid treated with T4, Zn, Se, and Vit.B12 (5.85 ± 1.91 µg/dl) as compared with normal control and hypothyroid groups.

Discussion

Body weight changes

In the hypothyroid group (Methimazole-treated rats group), after generating the hypothyroid animal model, we used different treatment strategies. The results showed a significant increase in the body weight of hypothyroid animals compared to the normal control group.

Table 2
Hormonal changes in control and treated groups (means \pm SD)

Groups	TSH (mIU/L)	T3 (ng/ml)	T4 (μ g/dl)
Control	0.50 \pm 0.10 a	1.11 \pm 0.09 a	4.18 \pm 1.05 a
Hypothyroidism	3.57 \pm 1.39 b	0.92 \pm 0.03 b	3.02 \pm 1.23 b
Hypothyroid+T4	1.28 \pm 0.81 ac	0.94 \pm 0.06 b	4.12 \pm 0.57. a b
Hypothyroid+IGF1	1.73 \pm 1.11 cd	1.02 \pm 0.13 ab	3.1 \pm 1.87 abc
Hypothyroid+T4+ IGF1	0.60 \pm 0.11 ae	1.10 \pm 0.21 a	3.54 \pm 1.36 ab
Hypothyroid+T4+Zn	1.87 \pm 0.01 cf	1.10 \pm 0.14 a	5.18 \pm 1.14 ade
Hypothyroid+T4+ Se	0.61 \pm 0.28 ae	1.08 \pm 0.15 abc	4.92 \pm 0.53 ade
Hypothyroid+T4+ B12	1.72 \pm 0.92 cdf	0.88 \pm 0.15 bd	4.06 \pm 0.52 ab
Hypothyroid+T4+Zn + Se+ B12	1.67 \pm 0.71 cdf	0.9 \pm 0.08 bd	5.85 \pm 1.91 e
Sig.	0.00	0.01	0.01

Different letters refer to a significant difference between the group. Similar letters refer to non-a significant differences between groups.

This finding is in accordance with previous studies. For instance, Sanyal and Raychaudhuri, (2016) showed that hypothyroidism is responsible for obesity. Overt hypothyroidism is associated with modest weight gain, but there is a lack of clarity regarding subclinical hypothyroidism. The changes in thyroid-stimulating hormone (TSH) could well be secondary to obesity. High leptin levels may play a role in the hyperthyrotropinemia of obesity and also increase susceptibility to thyroid autoimmunity and subsequent hypothyroidism (Sanyal and Raychaudhuri, 2016). In addition, Nayak and coworkers showed that the majority (61%) of patients with hypothyroidism were overweight (Nayak *et al.*, 2020). Also, these data showed that T4 and IGF1 treatment results in increasing the body weight however a combination of these materials caused a non-significant increase in the body weight compared to control. The exact mechanism of increasing body weight in response to IGF1 is not clearly understood however it has been shown that IGF1 induces the proliferation of target cells in various signaling pathways (Lin *et al.*, 2007).

Moreover, treatment of animals with T4, Zn, Se, and B12 alone or together non-significantly increased body weight. The results of the present study showed that this type of treatment may reduce the negative effect of body weight caused in the hypothyroid rats that produced a significant increase in body weight compared to the normal group. In this type of treatment decreases the adverse effect of hypothyroidism may be reduced by selenium and B12 supplementation. However,

Cavedon *et al.*, (2020) used 37 overweight/obese individuals aged 18–65 years, who adopted a slightly hypocaloric diet for 3 months. An intervention group received 240 µg/day of L-selenomethionine for 3 months; a control group received a placebo. Their results showed a significant change in body composition, involving a decrease in body fat mass, between the baseline and the end of the follow-up, in the intervention group. Unlike the placebo group, the group given Se had a significant increase in lean body and muscle mass and a significant decrease in leptin levels after 3 months of diet (Cavedon *et al.*, 2020).

Hormonal changes

In the hypothyroid group, the results of this study are inconsistent with a previous study that gave Methimazole as a 0.02 % solution in drinking water for three weeks induced a significant decline in T4 and T3 levels, as determined by radioimmunoassay (Cakic-Milosevic *et al.*, 2004). On the other hand, data of this study showed that the TSH level of the animal model of hypothyroid was significantly higher than that of normal control and IGF1 decreases the TSH of hypothyroid rats. The evidence of interdependence between G protein-coupled receptors and receptor tyrosine kinase signaling pathways has prompted a reevaluation of crosstalk between TSH and insulin-like growth factor-1 (IGF-1) receptor crosstalk, and its application to the clinic has in particular shown recent progress. Krieger *et al.*, (2020) summarized insights into the mechanism of TSH/IGF1 receptor crosstalk. They discussed evidence that crosstalk is one of the underlying causes of TSHR-based disease and the feasibility of using combinations of TSH receptor and IGF1 receptor antagonists to increase the therapeutic index for the treatment of Graves' hyperthyroidism and Graves' ophthalmopathy. Another studies indicated the close association between IGF and TSH (Krieger *et al.*, 2015, Krieger *et al.*, 2016, Krieger *et al.*, 2017). Also, Cerbo *et al.*, (2017) recorded the role of growth hormone (GH) and IGF-1 in thyroid function .

In addition, a significant decrease in TSH level in hypothyroid treated with T4+Zn group in comparisons with hypothyroid which considered a significant increase compared to normal groups. Also, there was a nonsignificant decrease in TSH level in hypothyroid treated with the T4+Se group compared to the normal group which was considered a significant decrease compared to the hypothyroid group. A previous study by Winther *et al.*, (2015) indicated that selenium is present in the active site of proteins important for thyroid hormone synthesis and metabolism. They investigated the effect of selenium supplementation in different doses on thyroid function, under conditions of suboptimal dietary selenium intake, they showed that plasma selenium concentrations increased significantly and dose-dependently in treatment groups receiving selenium while serum TSH and FT4 concentrations decreased significantly and dose-dependently. According to the previous study, T3 and T4 have an inverse relationship with TSH (Saravanan *et al.*, 2007), the data of this study also showed a similar pattern .

Moreover, zinc (Zn) and selenium (Se) are essential trace elements involved in thyroid hormone metabolism, and their supplementation affected the thyroid function of overweight or obese female hypothyroid patients. The mean serum FT3 increased significantly in the zinc & selenium and zinc groups ($p < 0.05$) but this

effect was significant in the zinc group compared to those in selenium ($p < 0.05$). Mean serum FT4 increased and TSH decreased significantly ($p < 0.05$) in the zinc & selenium group. They conclude some evidence of an effect of Zn alone or in combination with Se on thyroid function of overweight or obese female hypothyroid patients (Mahmoodianfard *et al.*, 2015). The role of Zn is complex and may affect both the synthesis and actions of the thyroid hormones (Hess *et al.*, 2010). The potential link between Zn and thyroid metabolism is based on the hypothesis that, like other nuclear receptors, T3 receptors contain nuclear Zn-binding proteins. Thus it seems that Zn is required for the biological function of thyroid hormones and related receptors. Zn deficiency has an inhibitory effect on thyroid hormones, whereas Zn supplementation has an opposite effect and is involved in the formation and mechanism of action of thyrotropin-releasing hormone (TRH), through a Zn-dependent process by carboxypeptidase, which converts prepro-TRH to TRH (Hammouda *et al.*, 2008).

The thyroid gland has the highest Se content and Se is inserted as selenocysteine, the 21st amino acid in the genetic code, into the families of selenoproteins, including glutathione peroxidase, thioredoxin reductase, and iodothyronine deiodinases (DIs). These contribute to thyroid hormone biosynthesis and metabolism, antioxidant defense, and control of the redox process in thyrocytes. Zn and Se are essential for the iodothyronine DI enzymes. DI selenoproteins are necessary for interconversion of T4 to its active metabolite T3 and inactive thyroid hormone products (Kohrle and Gartner, 2009). Moreover, severe and permanent Se deficiency associated with an impairment of thyroid hormone biosynthesis leads to the destruction of the thyroid follicles and their replacement with fibrous tissue (Kohrle and Gartner, 2009). An animal study showed no effect of Zn and Se supplementation on blood metabolic profile, except for the ratio of T4 and T3 hormones, which shows an important role of Se in converting T4 to T3 (Mudgal *et al.*, 2012).

Conclusion

According to our acknowledgment, to date, there was no specific study indicating the combination of T4 with IGF, T4, Zn, Se, and B12 treatments affects the thyroid gland and this study was the first. In this study, an animal model was used to investigate IGF-1 and some micronutrients including Se, Zn, and B12 in combination with T4. This study showed that these supplements improve the body weight changes and TSH, T3, and T4 in hypothyroid with fewer adverse effects compared to the hypothyroid group.

References

- Brent, G. A. 2012. Mechanisms of thyroid hormone action. *The Journal of clinical investigation*, 122: 3035-3043.
- Cakic-Milosevic, M., Korac, A. B. & Davidovic, V. S. (2004). Methimazole-induced hypothyroidism in rats: effects on body weight and histological characteristics of the thyroid gland. *Jugoslovenska medicinska biohemija*, 23:143-147.
- Cavedon, E., Manso, J., Negro, I., Censi, S., Serra, R., Busetto, L., Vettor, R., Plebani, M., Pezzani, R. & Nacamulli, D. (2020). Selenium Supplementation, Body Mass Composition, and Leptin Levels in Patients with Obesity on a

- Balanced Mildly Hypocaloric Diet: A Pilot Study. *International journal of endocrinology*, 2020: Article ID 4802739, 7 pages.
- Hammouda, F., Messaoudi I, El Hani J, Baati T, Saïd K, Kerkeni A. (2008). Reversal of cadmium-induced thyroid dysfunction by selenium, zinc, or their combination in the rat. *Biol Trace Elem Res* 126: 194–203.
- Hess, S.Y. (2010). The impact of common micronutrient deficiencies on iodine and thyroid metabolism: the evidence from human studies. *Best Pract Res Clin Endocrinol Metab* 24: 117–132.
- Kohrle, J. & Gartner, R. (2009). Selenium and thyroid. *Best Pract Res Clin Endocrinol Metab* 23: 815–827.
- Krieger, C. C., Neumann, S., Place, R. F., Marcus-Samuels, B. & Gershengorn, M. C. (2015). Bidirectional TSH and IGF-1 receptor cross-talk mediate stimulation of hyaluronan secretion by Graves' disease immunoglobins. *The Journal of Clinical Endocrinology & Metabolism*, 100: 1071-1077.
- Krieger, C. C., Place, R. F., Bevilacqua, C., Marcus - Samuels, B., Abel, B. S., Skarulis, M. C., Kahaly, G. J., Neumann, S. & Gershengorn, M. C. (2016). TSH/IGF-1 receptor cross-talk in Graves' ophthalmopathy pathogenesis. *The Journal of Clinical Endocrinology & Metabolism*, 101: 2340-2347.
- Krieger, C. C., Perry, J. D., Morgan, S. J., Kahaly, G. J. & Gershengorn, M. C. (2017). TSH/IGF-1 receptor cross-talk rapidly activates extracellular signal-regulated kinases in multiple cell types. *Endocrinology*, 158: 3676-3683.
- Krieger, C. C., Neumann, S. & Gershengorn, M. C. (2020). TSH/IGF1 receptor crosstalk: Mechanism and clinical implications. *Pharmacology & therapeutics*, 209: 107502.
- Lin, C. W., Yang, L. Y., Shen, S. C. & Chen, Y. C. (2007). IGF-I plus E2 induces proliferation via activation of ROS-dependent ERKs and JNKs in human breast carcinoma cells. *Journal of cellular physiology*, 212: 666-674.
- Mahmoodianfard, S., Vava, M., Golgiri, F., Khoshniat, M., Gohari, M., Solati, Z. & Djalali, M. (2015). Effects of zinc and selenium supplementation on thyroid function in overweight and obese hypothyroid female patients: a randomized double-blind controlled trial. *Journal of the American College of Nutrition*, 34: 391-399.
- Mohebbati, A. & Shaha, A. (2012). Anatomy of thyroid and parathyroid glands and neurovascular relations. *Clinical Anatomy*: 25, 19-31.
- Mudgal, V., Garg, A.K., Dass, R.S., Varshney, V. P. (2012). Effect of selenium, zinc, and copper supplementation on blood metabolic profile in 580 male buffalo (*Bubalus bubalis*) calves. *Biol Trace Elem Res* 145:304–311.
- Nayak, P. K., Mitera, S., Sahoo, J., Mahapatra, E., Agrawal, S. & Lone, Z. (2020). Relationship of subclinical hypothyroidism and obesity in polycystic ovarian syndrome patients. *Journal of family medicine and primary care*, 9:147.
- Nilsson, M. & Fagman, H. (2017). Development of the thyroid gland. *Development*, 144:2123-2140.
- Rousset, B., Dupuy, C., Miot, F. & Dumont, J. (2015). Thyroid hormone synthesis and secretion. Chapter 2 in Feingold KR, Anawalt B, Boyce A, *et al.*, editors South Dartmouth (MA).
- Sanyal, D. & Raychaudhuri, M. (2016). Hypothyroidism and obesity: An intriguing link. *Indian journal of endocrinology and metabolism*, 20: 554.
- Saravanan, P., Siddique, H., Simmons, D., Greenwood, R. & Dayan, C. M. (2007). Twenty-four hour hormone profiles of TSH, Free T3, and free T4 in hypothyroid

- patients on combined T3/T4 therapy. *Experimental and clinical endocrinology & diabetes*, 115: 261-267.
- Winther, K. H., Bonnema, S. J., Cold, F., Debrabant, B., Nybo, M., Cold, S. & Hegedus, L.(2015). Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population. *Eur J Endocrinol*, 172: 657-667.
- Yen, P. M. 2001. Physiological and molecular basis of thyroid hormone action. *Physiological reviews*, 81:1097-1142.