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Ultrastructure Study of Follicular Cells in Hypothyroidism-induced Rats Treated with Insulin-like Growth Factor-1 and Some Minerals

Douaa S. Hasoon[1], Maysaa A. Hadi[2]*, Ali M. Bahrami[1], Haider K. Zaidan[3]
1 Faculty of Veterinary Medicine, Ilam University, Ilam, Iran.
2 Department of Biology, College of Sciences, University of Babylon, Babylon, Iraq.
[3] Department of Medical Laboratories Techniques, College of Technology & Health Sciences , Al-Mustaqbal University, Babylon, Iraq.

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Abstract

The objective of this study is to examine the impact of the administration of levothyroxine (T4), insulin-like growth factor-1, zinc, selenium, and vitamin B12 for 2 months to methimazole-induced hypothyroid adult male Wistar albino eight-week-old rats weighing 180–250 gm were bought from the Ilam Lab of Animals and n=8 was for each group. The thyroid hormones, thyroid stimulating hormone, and ultrastructure of follicular cells by transmission electron microscope were compared between the control group and 7 groups of hypothyroid rats treated with T4, insulin-like growth factor-1, T4 + insulin-like growth factor-1, T4+zinc, T4+selenium, T4+ selenium, and T4+zinc +selenium + vitamin B12, respectively. The hypothyroid rats displayed a significant rise ($P \leq 0.05$) in thyroid-stimulating hormone levels compared with the control group, with significant differences verified in the treatment groups treated with GF1 in comparison to the hypothyroid group, while a significant difference in TSH levels was observed in hypothyroid rats administrated with T4+Zn, T4+Vit. B12, and T4+Zn+Se+Vit. B12 compared to those groups that were hypothyroid and control. Moreover, the methimazole-induced hypothyroidism group had ultrastructural degenerative alterations in both the cytoplasm and nucleus of the rat's thyroid follicular cells, while the treating groups had regeneration in the thyroid hormones, thyroid stimulating hormone, and ultrastructure of the follicular cells. In conclusion, both T4 + insulin-like growth factor-1 and T4 + zinc + selenium + vitamin B12 treatment groups produced a considerable enhancement in the thyroid follicular cell's ultrastructure and appropriate for application in hypothyroidism treatment.

Keywords: Hypothyroid Albino Rats; Insulin-Like Growth Factor-1(IGF-1); Levothyroxine (T4); Vitamin B12 (Vit. B12); Zinc (Zn) and Selenium (Se).

دراسة التركيب المستدق للخلايا الجريبية في الجرذان المستحث فيها نقص الدرقية المعاملة بعامل النمو المشابه للانسولين −1 وبعض المعادن

دعاء ساهي حسون[1], ميساء عادل هادي[2]*,علي محمد بهرامي[1], حيدركامل زيدان[3]
[1]كلية الطب البيطري, جامعة ايلام , ايلام, ايران
[2]قسم علوم الحياة, كلية العلوم, جامعة بابل, بابل, العراق
[3]قسم تقنيات المختبرات الطبية, كلية التقنيات والعلوم الصحية, جامعة المستقبل, محافظة بابل, العراق

*Email: mysadil2015@gmail.com

الخلاصة

ان هدف هذه الدراسة هو دراسة تأثير اعطاء هرمون الثايروكسين وعامل النمو المشابه للانسولين–1 والزنك والسيلينيوم وفيتامين B12 لمدة شهرين الى ذكور الجرذان البيضاء البالغة المصابة بنقص الغدة الدرقية المستحث بالميثيمازون والتي عمرها 8 اسابيع ووزنها 180–250 غرام والتي جهزت من البيت الحيولني في جامعة ايلام وعددها 8 جرذان لكل مجموعة. تمت مقارنة هرمونات الغدة الدرقية والهرمون المحفز للرقية والتركيب المستدق للخلايا الجريبية بالمجهر الالكتروني النافذ بين مجموعة السيطرة و7 مجاميع من الجرذان المصابة نقص الدرقية المستحث بالميثيمازون والمعاملة بالليفوثايروكسين، عامل النمو المشابه للانسولين–1، الليفوثايروكسين وعامل النمو المشابه للانسولين–1، الليفوثايروكسين والزنك ، الليفوثايروكسين والسيلينيوم ، الليفوثايروكسين وفيتامين B12، الليفوثايروكسين والزنك والسيلينيوم وفيتامين B12 على التوالي. اظهرت الجرذان المصابة بنقص الدرقية زيادة معنوية ($P\leq0.05$) في مستوى هرمون المحفز للدرقية مقارنة مع مجموعة السيطرة مع اثبات وجود اختلافات معنوية في المجاميع المعالجة بعامل النمو المشابه للانسولين– 1 مقارنة بالمجموعة المصابة نقص الدرقية مع وجود اختلافات معنوية في المجاميع المعالجة بالليفوثايروكسين والزنك ، الليفوثايروكسين وفيتامين B12، الليفوثايروكسين والزنك والسيلينيوم وفيتامين مقارنة بمجموعتي نقص الدرقية B12 والسيطرة. علاوة على ذلك، اظهرت المجموعة المستحث بها نقص الدرقية بالميثيمازون تغييرات تنكسية في التركيب المستدق لكلا من سايتوبلازم ونواة خلايا جريبات الغدة الدرقية في الجرذان بينما اظهرت مجاميع المعاملة التجديد في هرمونات الدرقية والهرمون المحفز الدرقي والتركيب المستدق للخلايا الجريبية. نستنتج بان كلا من المجموعتين المعاملة بالليفوثايروكسين وعامل النمو المشابه للانسولين–1 والمعاملة باليفوثايروكسين والزنك والسيلينيوم وفيتامين B12 انتجت تحسن ملحوظ في التركيب المستدق لخلايا جريبات الغدة الدرقية وهي ملائمة لتطبيقها في علاج نقص الدرقية.

1. Introduction

The thyroid gland is regulated by the hypothalamus and pituitary gland. The hypothalamus releases thyrotropin-releasing hormone (TRH) that stimulates the pituitary gland to release Thyroid-Stimulating Hormone (TSH). Under the normal conditions of hypothalamus and pituitary gland, they sense when the thyroid hormone levels are low, and they secrete more TRH and TSH that stimulates to make more thyroid hormones and vice versa. More hormones are produced in the body when it is needed[1]. Thyroid hormones, triiodothyronine (T3) and thyroxine (T4) are made by the follicle-containing thyroid gland [2]. There are five fundamental processes in the manufacturing of thyroid hormones, comprising the production of the glycoprotein thyroglobulin (TG), uptake of iodide, thyroglobulin iodination, storage, and discharge. Moreover, T3 and T4 production are reduced by an iodine shortage in the body. These two hormone deficiencies and excesses cause major diseases like hypothyroidism and hyperthyroidism [1].

A very widespread condition in global health is hypothyroidism [3,4], which can considerably improve mitochondrial respiratory chain dysfunction, giving rise to the accelerated formation of free radicals that later generate oxidative stress [4]. The underlying biological processes that lead to hypothyroidism have been thoroughly investigated in laboratory animal models, providing a comprehensive understanding of the condition's fundamental mechanisms. Additionally, prophylactic and therapeutic effects were evaluated using these models [5]. The conventional method of treatment includes both medical therapies like synthetic sodium levothyroxine and surgical interventions like thyroidectomy, which will both lead to comorbid disorders and adverse effects such as hormonal abnormalities, juvenile diabetes, and hypocalcemia. Almost everybody's cells are impacted by thyroid hormones. They accomplish this by interfering with protein synthesis, raising the basal metabolic rate, and controlling the breakdown of lipids, proteins, and carbohydrates [6].

The main growth hormone (GH) mediator is insulin-like growth factor-1 (IGF-1), which is crucial to stimulating childhood cell development and differentiation and has anabolic effects on adults [7,8]. Both zinc (Zn) and selenium (Se), trace elements, are essential and fundamental for thyroid hormone metabolism. Generally, zinc has a complicated interaction with thyroid hormones and is recognized as regulating these hormones' production and mode of action, which engages in thyroid hormone activity and metabolism [9,10]. Additionally, individuals with autoimmune thyroid disease have often been found to have vitamin deficiencies, B12 deficiency (Vit. B12 deficiency). Vit-B12 deficiency was associated with autoimmune hypothyroidism, and that there was a negative correlation between vit-B12 levels and anti-TPO antibodies in these patients with autoimmune hypothyroidism, and vit-B12 deficiency should be investigated at the time of diagnosis and periodically on follow-ups [11.12]. Therefore, the purpose of this research was to clarifying the effects of levothyroxine (T4), IGF-1, Zn, Se, and Vit. B12 on thyroid follicular cells in hypothyroid rats. Also, to follow up the alterations in cytology and ultrastructure of the rat's thyroid follicular cells after T4, IGF-1, Zn, Se, and Vit-B12 therapy in hypothyroid rats of different groups.

2. Materials and Methods

2.1 Materials

2.1.1 Solution of Methimazole

Methimazole (Sigma Aldrich, USA) was administered in the drinking water at a concentration of 60 mg/kg/day for a period of three weeks cause a big drop in T3 and T4 levels [13].

2.1.2 Solution of Levothyroxine

Levothyroxine (LT4) was administered as levothyroxine sodium (Merck Healthcare, Germany) at a dose of 1.6 g/kg body weight [14].

2.1.3 Solution of IGF-1

The recombinant human IGF-I (Pharmacia-Upshon, Sweden) solution at a dosage of 2 µg/100 g/day was used [15].

2.1.4 Solutions of Zinc and Selenium

Zinc-gluconate (Webber Naturals, Canada) was used to prepare a solution containing 30 mg of zinc, whereas the solution of Se, which contained 200 mg of selenium (Webber Naturals, Canada) was utilized [16].

2.1.5 Solution of Vitamin B12

The dosage of Vit. B12 solution was (500 µg /day) (Casasco Laboratories, Argentina) [17].

2.2 Experimental Animals

Male Wistar albino eight-week-old rats weighing 180–250 gm were bought from the Ilam Lab of Animals (Ilam, Iran) and were kept separately in lab cages. Prior to initiation of the studies, the rats underwent a 7-day adaptation period in housing facilities. They were provided food and water *ad libitum*. After the adaptation period, the rats were used for the experimental studies.. The temperature was held steady at 22.2-23.3°C. From 7:00 AM to 7:00 PM, the lights were on, and from 7:00 PM to 7:00 AM, they were off. The procedures have received approval from the Institutional Animal Care and Use Committee (IACUC) of Ilam University according the administrative order No. 220466 on 15/2/2021.

2.3 Experimental design

Normal and hypothyroid rats were randomly divided to 9 groups (n=8). After the animals were sacrificed, hormones and ultrastructure changes of thyrocytes by transmission electron microscope (TEM) were investigated. The study groups were organized as follows:

1- The control group contained normal rats and distilled water was administered orally.

2-The hypothyroid group: comprised of 8 rats provided a solution of methimazole (MMI) (dosage of 60 mg/kg/day for 3 weeks), an antithyroid medication, orally to achieve a significant decline in T3 and T4 levels [13].
3-Treatment groups: The 56 rats were administered a solution of methimazole as a hypothyroid group [13] then separated into seven subgroups (n=8) and treated orally with various methods for 2 months as follows:
1. The first subgroup was administered T4.
2. The second subgroup was administered with IGF-1.
3. The third subgroup was administered with T4+IGF-1.
4. The four subgroups were administered with T4+Zinc.
5. The fifth subgroup was administered with T4 + Selenium.
6. The sixth subgroup was administered with T4+Vit. B12.
7. The seventh subgroup was administered with T4+ Zinc+ Selenium+ Vit. B12.

2.4 Blood Sampling

The rat is severely anesthetized at the end of the experiment. During the procedure, a piece of cotton immersed in the anesthetic chloroform was placed in a desiccator to ensure sustained anesthesia. A heart puncture was used to take blood, which was drawn using a sterile syringe. Following collection, blood was centrifuged, and serum was obtained to evaluate the levels of TSH, T4, and T3 hormones using the ELISA technique.

2.5 The Quantitative Analysis of Triiodothyronine, Thyroxine, and Thyrotropin Levels

Levels of total T3 and T4 in rat serum were measured by the Pishgaman Sanjesh kit (Iran), while thyrotropin (TSH) levels were quantitatively determined using the Calbiotech kit (USA), following the guidelines provided by the manufacturers.

2.6 Preparation of the Thyroid Gland for Transmission Electron Microscopy

The thyroid gland was removed carefully and prefixed with 2.5% glutaraldehyde for two hours at 4 °C. Then, in the PBS (0.1 M phosphate buffer saline, pH 7.2), samples were rinsed three times (10 minutes each). Following rinsing, the samples were post-fixed in 1% osmium tetroxide (TAAB Laboratories Equipment, England) for 1 hour at room temperature. After three PBS washes (10 minutes each), the samples were dehydrated in a series of ascending alcohol, acetone, and acetone-resin mixture (50/50). The final step was embedding in TAAB embedding resin (TAAB Laboratories Equipment, England), followed by 48 hours of polymerization at 60 °C. Then, semi-thick slices (0.5-1μ) stained with methylene blue and sections of ultra-thin (50 nm) were created by Leica Ultracut R (Leica, Wetzlar, Germany), placed on a 300 mesh copper grid, and double-stained with 20% uranyl acetate (BDH Laboratory Chemicals, England) in pure methanol for 45 minutes and in Reynolds solution (lead nitrate and sodium citrate) [18]. Finally, a TEM (Philips, Nederland) examination of the sections was performed at a voltage of 100 KV.

2.7 Statistical analysis

Based upon an ANOVA analysis of the impacts of each treatment on T3, T4, and TSH, statistical analyses were performed by applying SPSS software (Version 21). Also, $P \leq 0.05$ was used to determine whether the mean differences were significant.

3. Results

3.1 Hormonal assay

In Figure 1, the findings demonstrated a significant rise ($P \leq 0.05$) in the TSH levels of rats with hypothyroidism in comparison with the normal control, while TSH levels had a non-significant decline in hypothyroid rats administrated with T4, T4+IGF-1, and T4+Se in

comparison with the normal group, whereas they showed a significant drop when compared to the hypothyroid group. The hypothyroid group treated with IGF-1 showed a significant decrease in TSH levels compared to the untreated hypothyroid group, while a significant difference in TSH levels was observed in hypothyroid rats administrated with T4+Zn, T4+Vit. B12, and T4+Zn+Se+Vit. B12 compared to those groups that were hypothyroid and normal.

Also, there was a significant decline in T3 levels in hypothyroid and hypothyroid administered with the T4 group in comparison with the normal control group. The nonsignificant variation was recorded in T3 levels in all hypothyroid-treated groups in comparison with the normal control group but was still viewed as a significant rise when compared to the hypothyroid group. Moreover, T4 levels significantly declined in the hypothyroid group in comparison with the normal control group, although there were no significant differences in T4 levels in all hypothyroid-treated groups in comparison with the hypothyroid and normal control groups. Also, a significant increment in T4 levels in hypothyroid was administered with T4+Zn, T4+Se, and T4+ Zn+ Se+ Vit. B12 groups as compared with the normal control and hypothyroid groups.

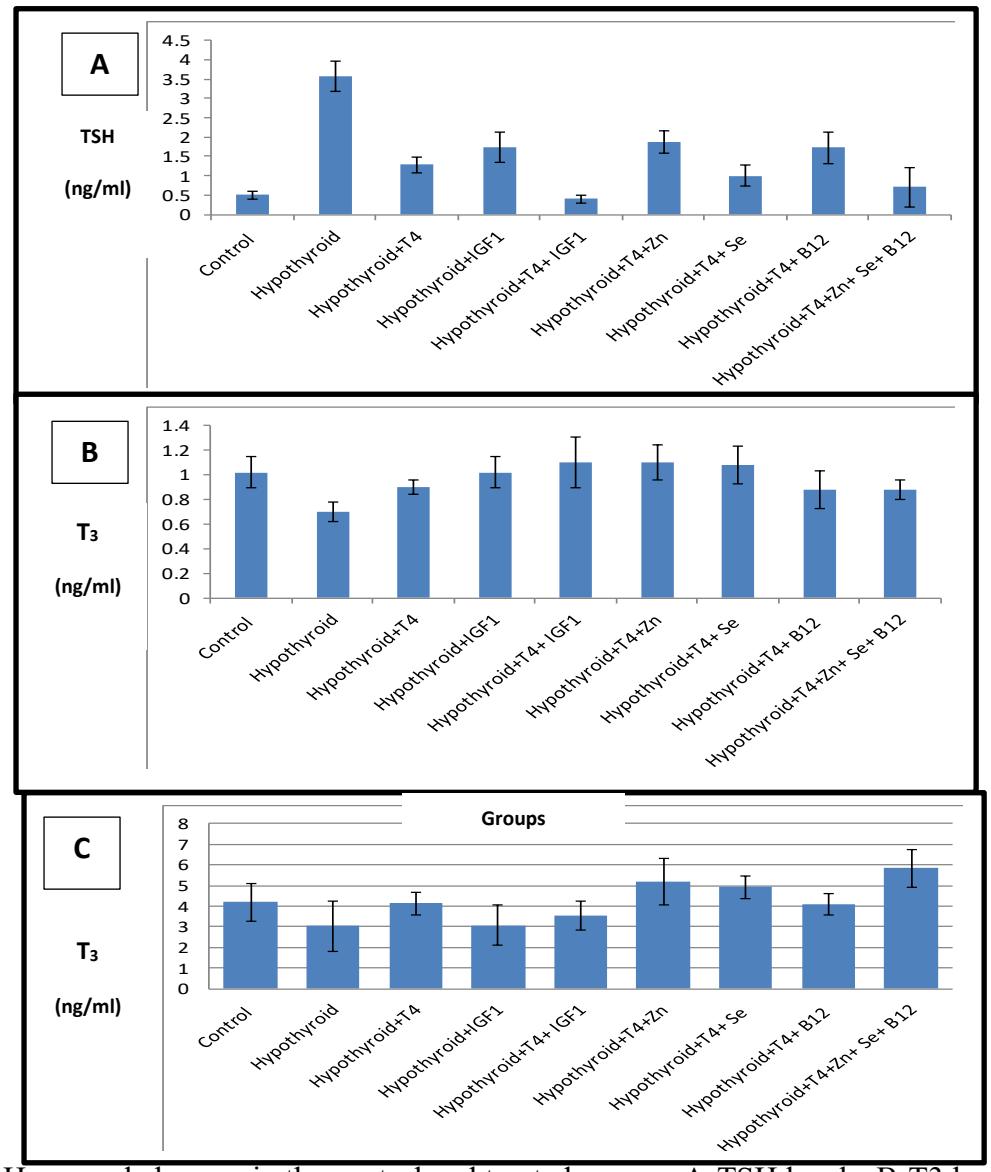

Figure 1: Hormonal changes in the control and treated groups. A-TSH levels, B-T3 levels, C-T4 levels.

3.2 Cytological study and ultrastructure changes in thyroid follicular cells *3.2.1 Control Group*

In the control group, TEM sections of the thyroid gland showed that the follicular cells (thyrocytes) possessed a rounded euchromatic nucleus with a regular nuclear membrane and a well-formed and prominent nucleolus. The follicular cell displayed an apical border with protruding microvilli in the direction of the lumen of follicular cells (Figure 2A–B). Ultrastructurally, most organelles of the control group can be found in the cytoplasm of normal thyrocytes which has plentiful paralleled cisternae of well-developed rough endoplasmic reticulum, colloid droplets, the Golgi complex, and mitochondria (Figure 2C).

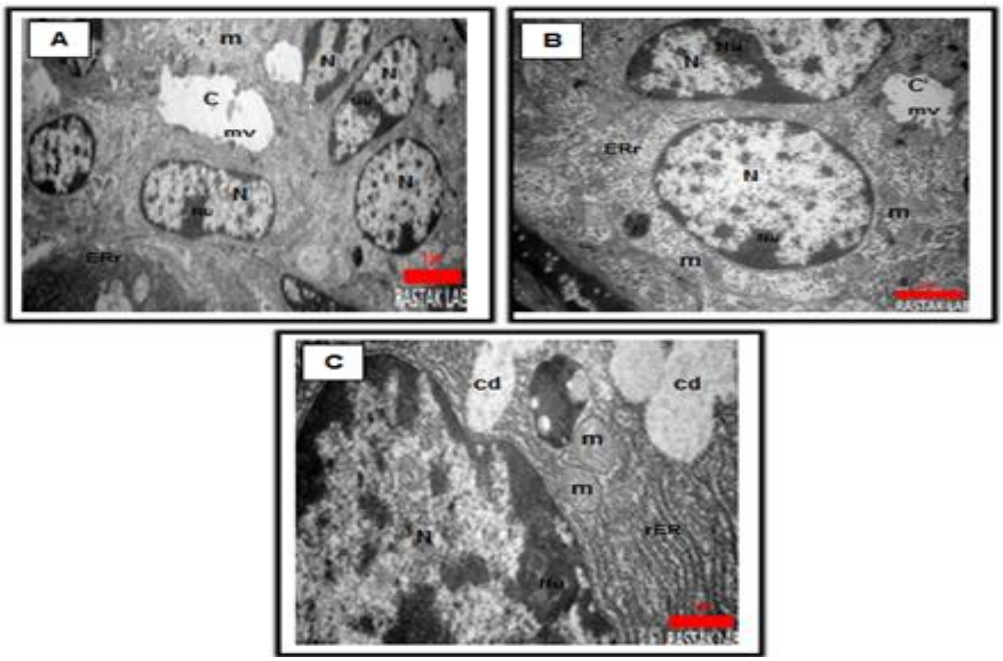

Figure 2: Thyroid follicular cells in a micrograph taken by TEM in a control group. A, B) Follicular cells had rounded euchromatic nuclei (N) with the regular nuclear membrane and prominent nucleolus (Nu). The follicular cell's ultrastructure revealed an apical border with protruding microvilli (mv) and follicles filled with colloid (C) C). The cytoplasm has plentiful paralleled cisternae of the endoplasmic reticulum (rER), colloid droplets (cd), and mitochondria (m).

3.2 Hypothyroid Group

The ultrastructure of thyroids in methimazole-treated rats revealed that the methimazole-induced ultrastructural alterations in rat follicular cells resulted in degenerative changes in both the cytoplasm and nucleus of most follicular cells as a marked decrease in the rough endoplasmic reticulum and mitochondria with the marked presence of vacuoles and debris in the cytoplasm (Figure 3A-B). Further alterations of follicular cells in the hypothyroid group comprise degenerative changes, such as shrunken, irregular-shaped nuclei with increased amounts of heterochromatin visible (Figure 3C). This study revealed nuclei with irregular shapes and increased aggregation of peripheral heterochromatic chromatin, accompanied by the presence of vacuolated cytoplasm (Figure 3A, B, and C).

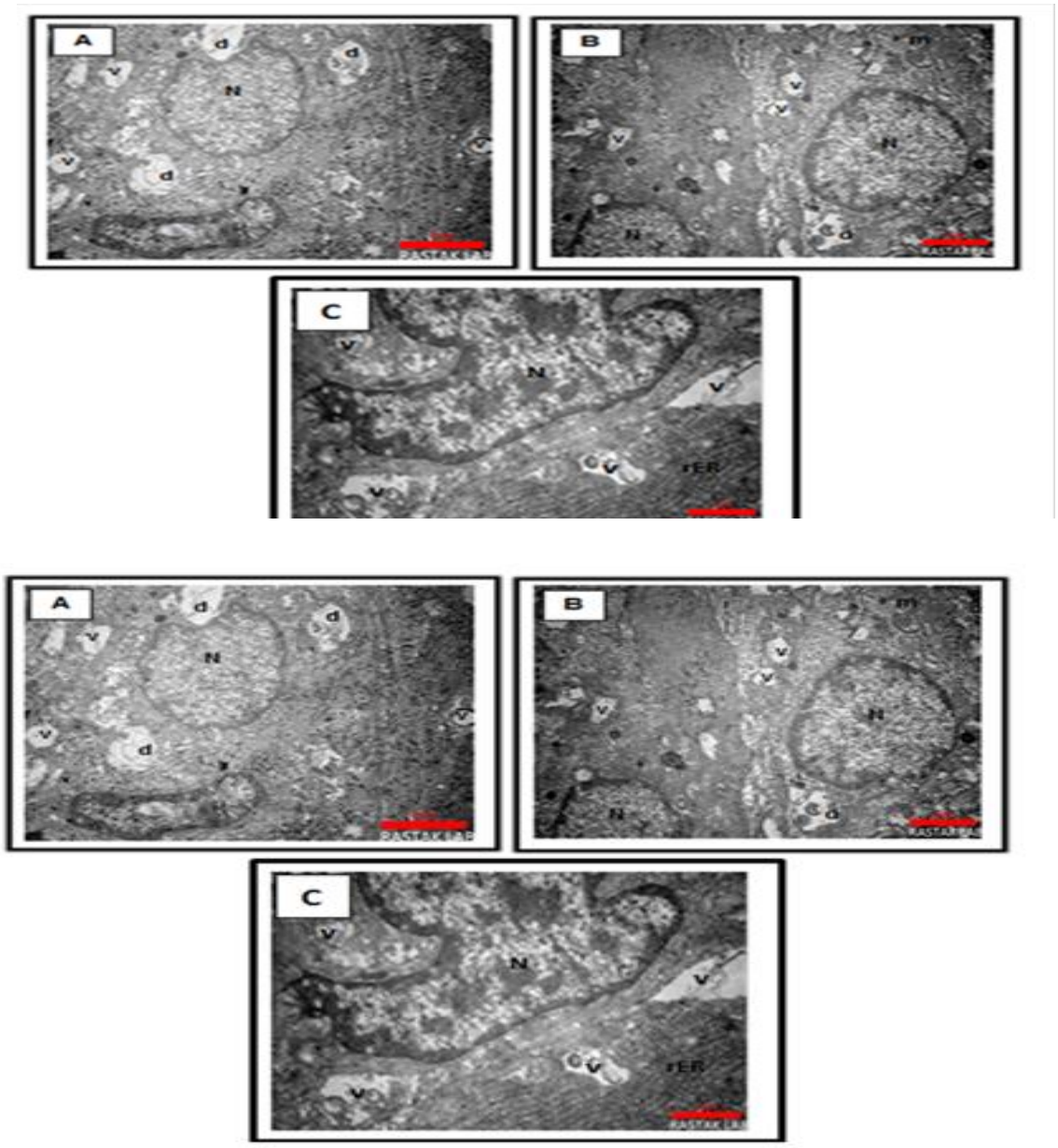

Figure 3: Thyroid follicular cells in a micrograph taken by TEM in the methimazole-induced hypothyroid group. A&B) Degenerative changes of cytoplasm and nuclei with the decrease in the rough endoplasmic reticulum (rER) and mitochondria (m) in addition to the marked presence of vacuoles (v) and cellular debris (d) in the cytoplasm of follicular cells. C) Nucleus with increased amounts of heterochromatin and a marked presence of vacuoles (v) in the cytoplasm of follicular cells.

3.3 Hypothyroidism Treated with the T4 Group

Some follicular cells became marked with signs of regeneration as well as developed rough endoplasmic reticulum with an increased number of mitochondria, while the cytoplasm still had a sign of degeneration as the presence of vacuoles in the cytoplasm (Figure 4A-B), while another follicular cell still had degenerative changes as nuclei with condensed chromatin, and the presence of vacuolated cytoplasm (Figure 4C).

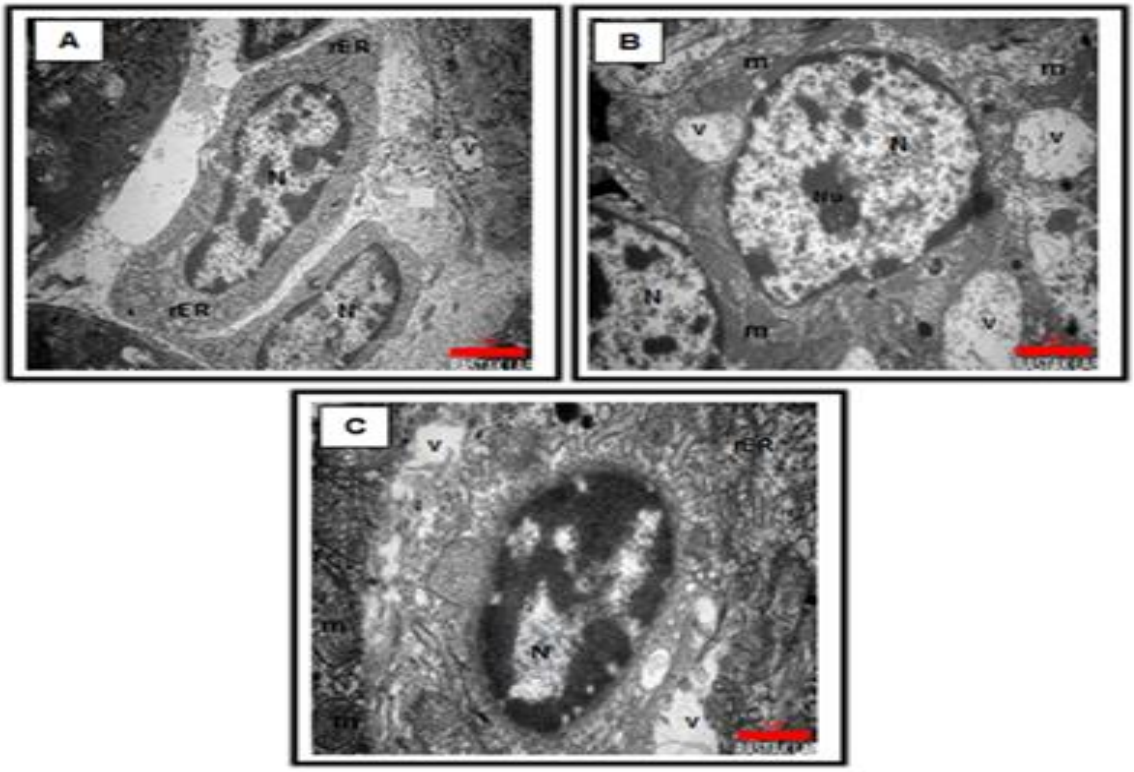

Figure 4: Thyroid follicular cells in a micrograph taken by TEM in hypothyroid rats treated with T4. A, B) Some follicular cells appeared with signs of regeneration such as normal and regular-shaped nuclei (N) and nucleoli (Nu) with a well-developed rough endoplasmic reticulum (rER), increased number of mitochondria (m) but vacuoles (v) still present in the cytoplasm. C) Some follicular cells still had degenerative changes as nuclei (N) with condensed chromatin, the presence of vacuolated cytoplasm (v) but regenerated endoplasmic reticulum (rER) was clear.

3.4 Hypothyroidism Treated with IGF-1 Group

In hypothyroid rats treated with IGF-1, regeneration was observed in most follicular cells. In addition, an increase in well-developed rough endoplasmic reticulum with an increasing number of mitochondria and colloid droplet formation was markedly observed (Figure 5A–B), and normal nuclei with nucleolus were observed (Figure 5C).

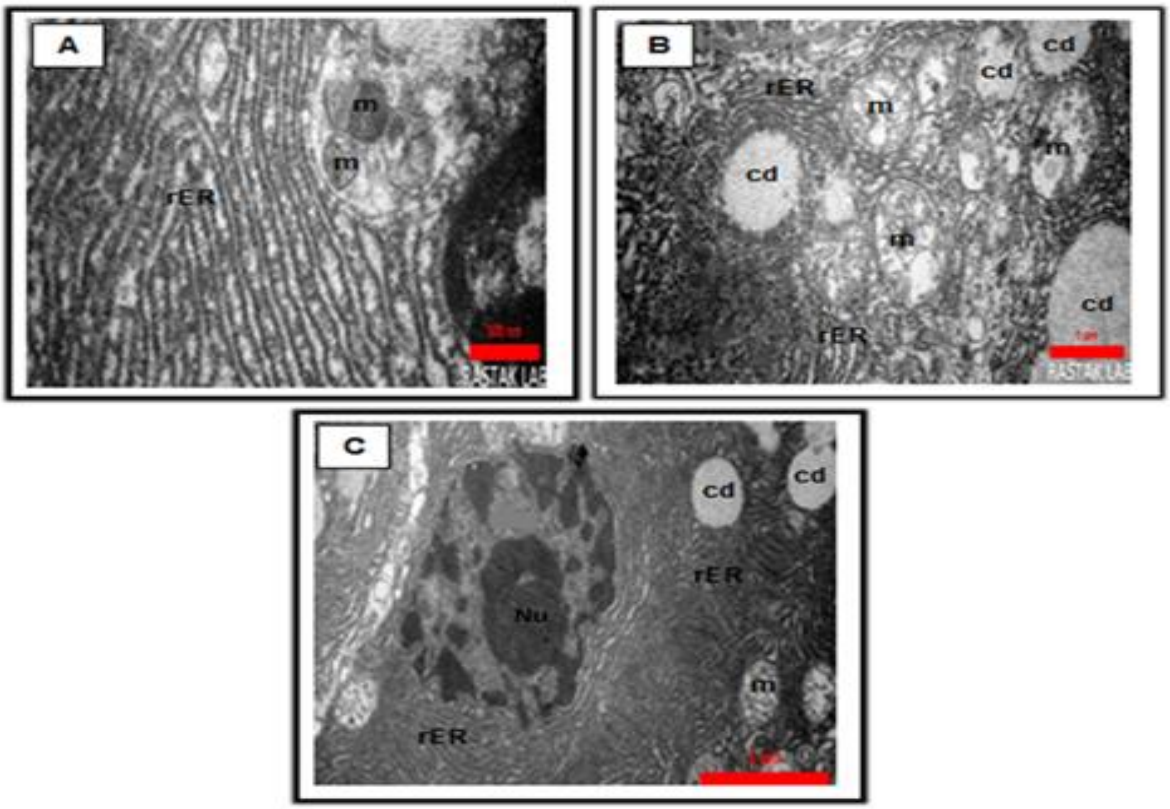

Figure 5: Thyroid follicular cells in a micrograph taken by TEM in hypothyroid rats treated with IGF-1. A, B) Follicular cells appeared to have an increasingly well-developed rough endoplasmic reticulum (rER) with an increasing number of mitochondria (m) and colloid droplets (cd) of various diameters in the cytoplasm. C) Other follicular cells had a clear nucleolus (Nu), with an increasingly well-developed rough endoplasmic reticulum (rER), mitochondria, and colloid droplet formation markedly observed.

3.5 Hypothyroidism Treated with T4 and IGF-1 Group

Most of the follicular cells possessed an euchromatic nucleus that had a regular and rounded shape, a prominent nucleolus, and a well-formed nuclear membrane. This group showed one of the most effective regenerations of follicular cells, closely resembling the control group. Ultrastructurally, most organelles available in the cytoplasm of normal follicular cells, which have plentiful mitochondria, paralleled cisternae of well-developed rER, and colloid droplets of various diameters in the cytoplasm of the follicular cell (Figure 6A–B).

3.6 Hypothyroidism Treated with T4 and the Zn Group

In this group, some follicular cells had normal nuclei, with the existence of an increased number of mitochondria and a rough endoplasmic reticulum but vacuolated cytoplasm still present (Figure 7A–B).

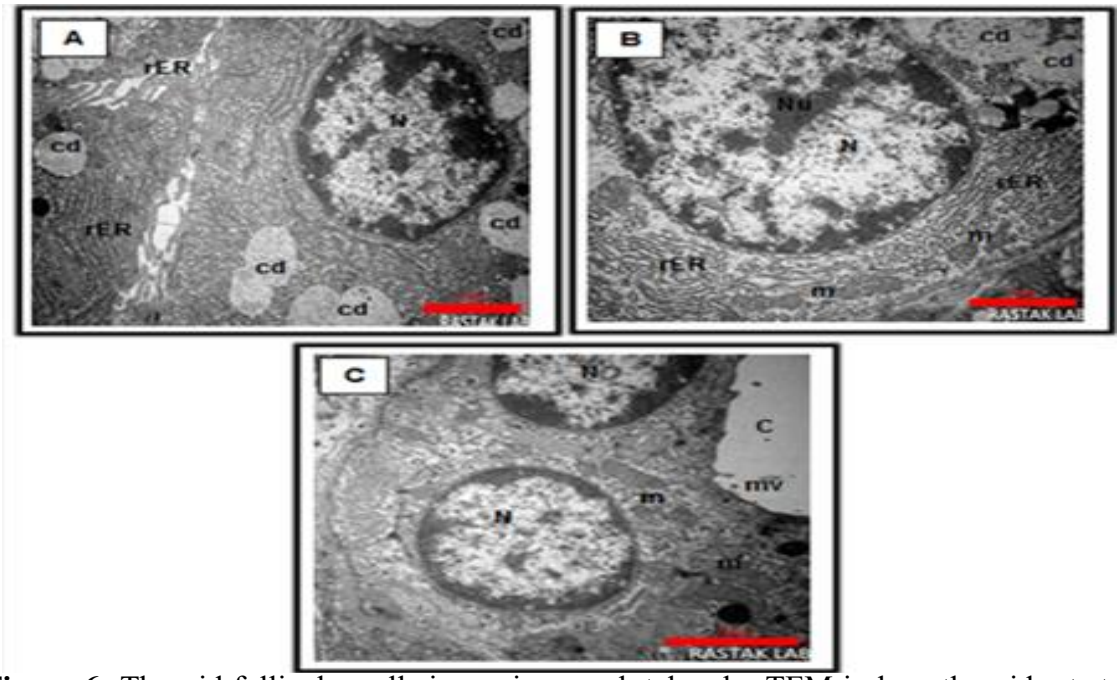

Figure 6: Thyroid follicular cells in a micrograph taken by TEM in hypothyroid rats treated with T4 and IGF-1. A, B) The normal regular nucleus with nucleolus (Nu) and most organelles can be found in the cytoplasm as mitochondria (m), the well-developed rough endoplasmic reticulum (rER), and colloid droplets are detectable (cd). C) At the apical border, follicular cells had regular microvilli (mv) protruding into the follicular lumen containing colloid (C).

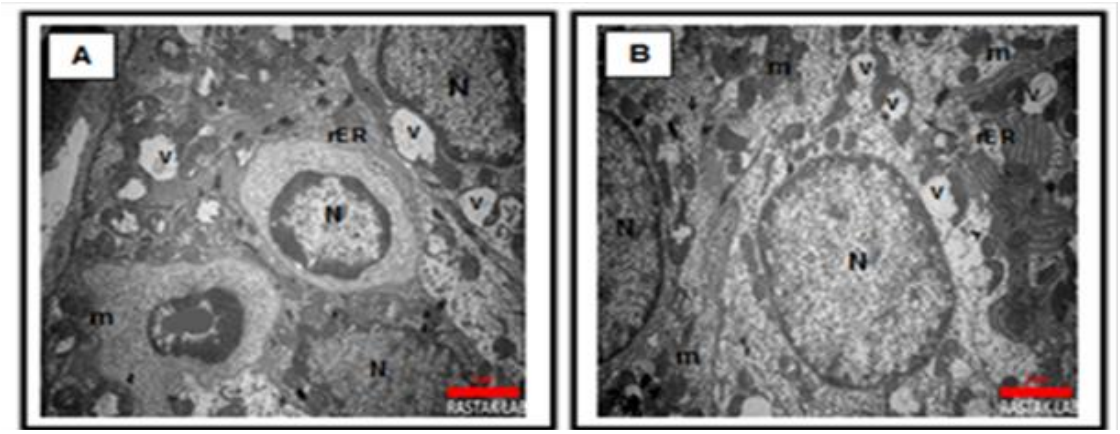

Figure 7: Thyroid follicular cells in a micrograph taken by TEM micrograph in hypothyroid rats treated with T4+Zn. A, B) Some follicular cells had normal nuclei appearance with the presence of numerous mitochondria (m) and a well-developed rough endoplasmic reticulum (rER) but vacuolated cytoplasm was still present.

3.7 Hypothyroidism Treated with the T4+Se Group

In hypothyroid rats administered with T4 and Se, most follicular cells had normal nuclei appearance with the presence of mitochondria (Figure 8A) and well-developed rER with the presence of numerous colloid droplets with various diameters in the cytoplasm (Figure 8B), however, but some degenerated nuclei with heterochromatin condensation and an irregular plasma membrane were still seen (Figure 8C).

3.8 Hypothyroidism Treated with T4 and Vit. B12 Group

Treatment of hypothyroid rats with T4+Vit. B12 resulted in the regeneration of most follicular cells, which regenerated near the normal control group with normal regular nuclei and numerous mitochondria (Figure 9A). Large colloids with increased microvilli were observed in addition to fewer degenerated nuclei (Figure 9B). The presence of mitochondria with normally arranged cristae returns close to that of the normal control group (Figure 9C)

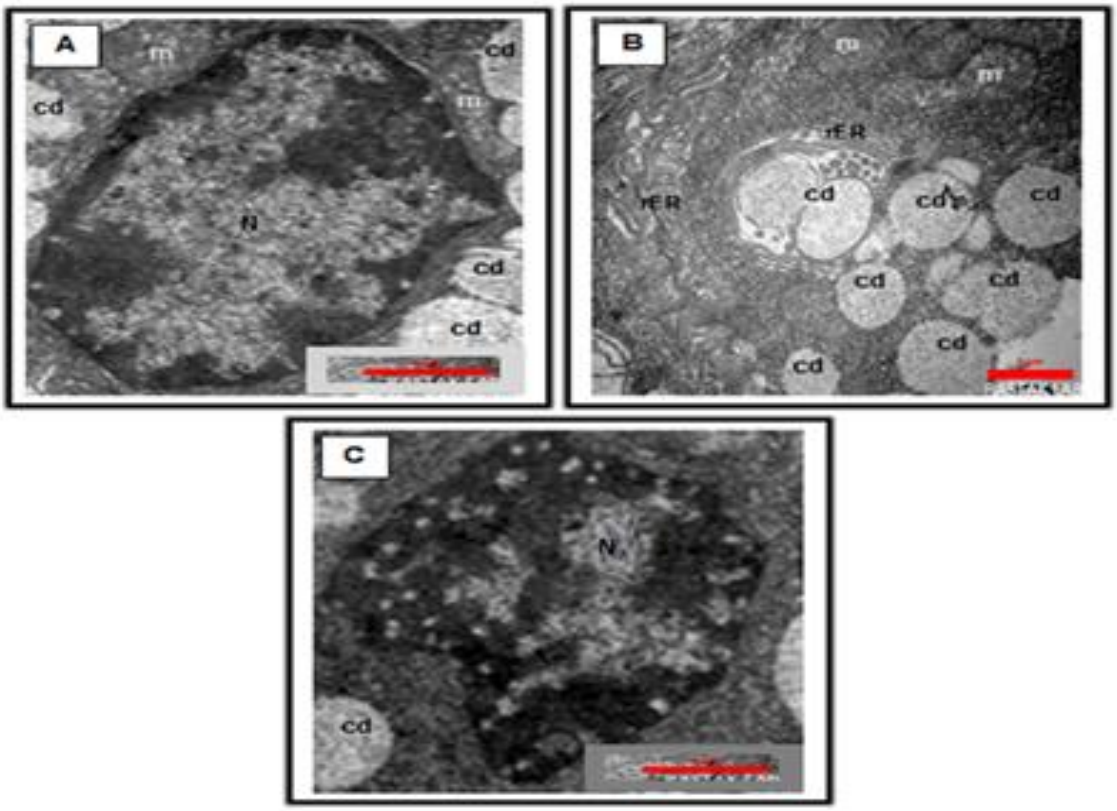

Figure 8: Thyroid follicular cells in a micrograph taken by transmission electron micrograph in hypothyroid rats treated with T4+Se. A, B) Most follicular cells had normal nuclei (N) appearance with the presence of mitochondria (m) and a well-developed rough endoplasmic reticulum (rER) with the presence of numerous colloid droplets (cd) had various diameters in the cytoplasm. C) Nucleus with chromatin condensation and an irregular plasma membrane still visible.

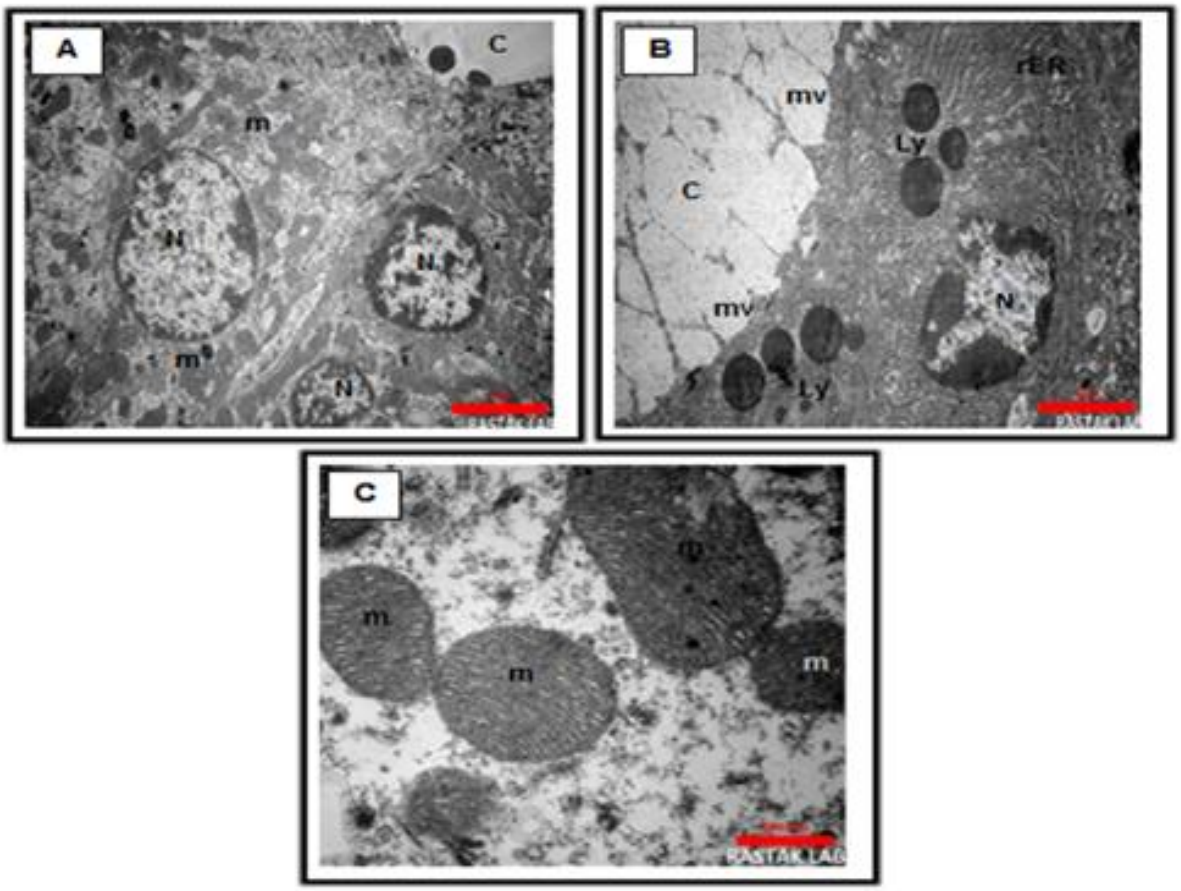

Figure 9: Thyroid follicular cells in a micrograph taken by TEM micrograph in hypothyroid rats administrated with T4+Vit.B12. A) Most follicular cells regenerated and reversed near the normal control group with a normal regular nucleus and a large number of mitochondria. B) At the apical pole of follicular cells, large colloids with increased microvilli (mv) were observed in addition to fewer degenerated nuclei with the presence of lysosomes (Ly). C) Presence of mitochondria with normally arranged cristae return close to the normal control group.

3.9 Hypothyroidism Treated with T4, Zn, Se, and Vit.B12 Group

In this group, most follicular cells regenerated close to the normal control group with a normal regular nucleus, a large number of mitochondria, and a well-developed rough endoplasmic reticulum (Figure 10A-B). Increased colloid droplets were observed (Figure 10C). In addition, no degenerated nuclei were seen.

4. Discussion

The current results recorded that TSH levels had a non-significant decline in hypothyroid rats administered with T4, T4+IGF-1, and T4+Se in comparison with the normal group, whereas they showed a significant drop when compared to the hypothyroid group. The most favorable results were observed in IGF-1, T4, zinc, Se, and Vit B12. Levothyroxine(LT4) replacement therapy is the mainstay of treatment for hypothyroidism to restore clinical and biochemical euthyroidism[19,20].

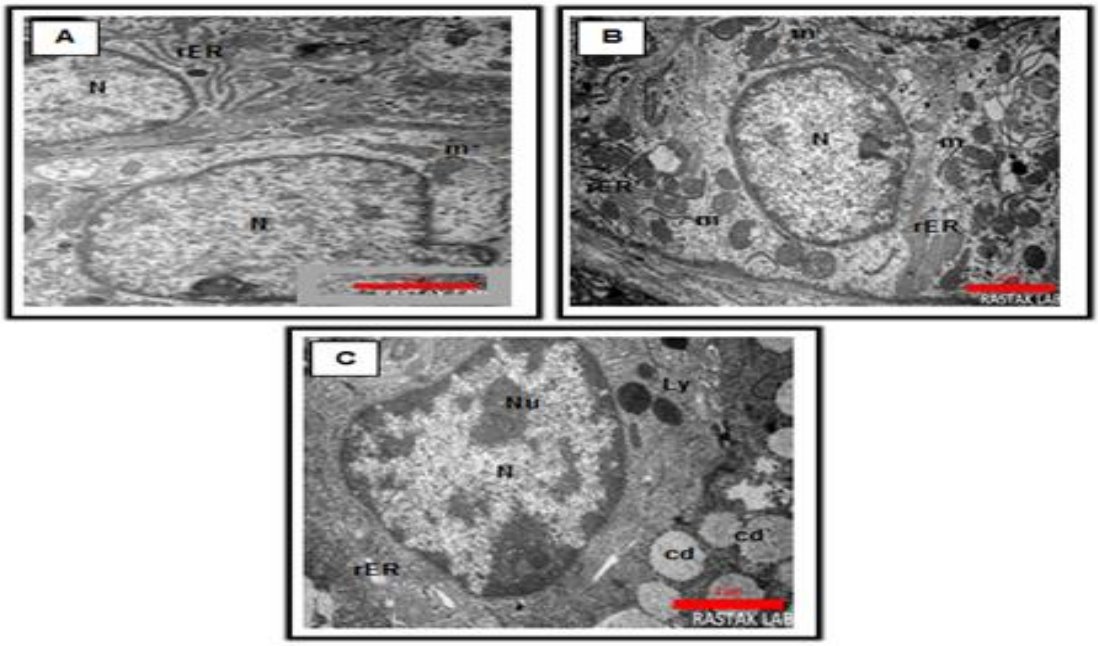

Figure 10: Thyroid follicular cells in a micrograph taken by a transmission electron micrograph in hypothyroid rats administrated with T4, Zn, Se, and Vit.B12. A, B) Most follicular cells regenerated near the normal control group with normal regular nuclei (N) with nucleolus (Nu) and a large number of mitochondria (m) and well-developed rough endoplasmic reticulum (rER). C) Follicular cells with increased colloid droplets were seen.

The recent study displayed that the hypothyroid rats had significant rise ($P \leq 0.05$) in thyroid-stimulating hormone levels compared with the control group, with significant differences verified in the treatment groups treated with GF1 in comparison to the hypothyroid group, while a significant difference in TSH levels was observed in hypothyroid rats administrated with T4+Zn, T4+Vit. B12, and T4+Zn+Se+Vit. B12 compared to those groups that were hypothyroid and control. The data from earlier studies showed that 35–60% of patients treated fail to reach target TSH levels and prove to be either undertreated or overtreated clearly with negative consequences [19,21]. Regarding this, the chance that excessive treatment could result in exogenous subclinical hyperthyroidism with potential cardiovascular complications is one of the most important issues in hypothyroidism treatment, particularly in the elderly [22,23,24]. Thyroglobulin has an aggregated interior in the follicle and is implicated in controlling the activity of follicular cells by balancing the effects of TSH [25]. Elevated pressure on follicular cells caused by the exaggerated intrafollicular aggregation of thyroglobulin may cause cytoskeleton deformation and the production of pro-apoptotic signals [26].

The cytological study revealed some ultrastructure changes in follicular cells. Ultrastructurally, most organelles of the control group can be found in the cytoplasm of normal thyrocytes which has plentiful paralleled cisternae of well-developed rough endoplasmic reticulum, colloid droplets, the Golgi complex, and mitochondria (Figure 2C). Following the previous investigation, electron microscopy showed that follicular cells from the control group had the Golgi complex, endoplasmic reticulum, prominent nucleus, and colloid droplets. Follicular cells contained several regular and long microvilli that extended into the apical surface of follicular cells [26].

The ultrastructure of thyroids in methimazole-treated rats revealed that the methimazole-induced ultrastructural alterations in rat follicular cells were degenerative changes in both the cytoplasm and nucleus of most follicular cells, as indicated by a significant reduction in the rough endoplasmic reticulum and mitochondria with the marked presence of vacuoles and debris in the cytoplasm. This result may be due to the fact that methimazole may generate an antioxidant imbalance, as reported previously [13]. Also, methimazole accelerates free creation and decreases the capability of antioxidative protection; thus, it is accompanied by oxidative stress [27]. A disequilibrium between oxidants and antioxidants results in cellular damage. In addition, activated oxidant systems result in large amounts of reactive nitrogen species and reactive oxygen species (ROS), nitration, glutathionylation or carbonylation of proteins, lipid peroxidation, and DNA fragmentation [28].

In the hypothyroidism group treated with T4, some follicular cells were marked with signs of regeneration and developed a rough endoplasmic reticulum with an increased number of mitochondria, but the cytoplasm still had a sign of degeneration, such as the presence of vacuoles in the cytoplasm. This result may be because that the thyroid metabolic hormone controls the rate of metabolism. The association between thyroid dysfunction and body mass index (BMI) at baseline and after normalization of the hormone levels was evaluated. Normalization of thyroid levels significantly changed the weight of patients, but remaining most patients within overweight ranges[29]. The metabolism of minerals and bone strength are both significantly impacted by thyroid hormones. In hypothyroid women, total thyroidectomy can result in a significant rise in body mass index (BMI) and a significant decline in thyroid hormones, osteopontin, and levels of calcium [30]. Despite the T4 treatment providing some protection from the methimazole-induced rise in oxidative stress, other studies show that 35 to 60 percent of patients on T4 treatment fail to achieve the desired TSH levels and are either undertreated or overtreated, both of which have detrimental effects [19,21]. Thyroglobulin, which has an aggregated structure within the follicle, plays a role in regulating the activity of follicular cells by modulating the effects of TSH [25]. Elevated pressure on follicular cells caused by the exaggerated intrafollicular aggregation of thyroglobulin may cause the deformation of the cytoskeleton and the production of pro-apoptotic signals [26].

In hypothyroidism rats treated with the IGF-1 group, there hasn't yet been any study that specifically demonstrates the IGF-1 treatment effect on thyroid gland ultrastructure. However, IGF-I is a key regulator of the functions, growth, and development of numerous human tissues [27]. This study's findings indicated that the thyroid gland of rats treated with IGF-1 was seen to have more active follicles and was full of colloidal fluid than the hypothyroidism group. The IGF-1 treatment positively affected the thyroid gland's ultrastructure. However, IGF-I is a key regulator of the functions, growth, and development of numerous human tissues. Further, IGF-I and growth hormone's biological impact on target tissues can also be influenced by thyroid hormones and thyrotropin (TSH) [27].

The hypothyroidism group treated with T4 and IGF-1 was one of the best groups in the regeneration of follicular cells and reverted closely to the control group. Specifically, IGF-I supports the thyroid gland to maintain normal volume, function, and hormone production. While some of these effects may be mediated by increased sensitivity to TSH's effects, others may not depend on pituitary function. Also, IGF-I contributes to pathological thyroid disorders, such as benign tumorigenesis and enlargement seen in acromegaly [28].

In hypothyroid treated with T4+Zn and T4+Se groups, thyroid hormone activity and metabolism involve different nutrients, such as zinc, which has a complicated relationship with

thyroid hormones and is recognized as regulating these hormones' production and mode of action. The vital trace minerals zinc and selenium, both of which play crucial roles in the metabolism of thyroid hormones [9, 10].

Strong evidence suggests that vitamin B12 deficiency is more prevalent among patients with thyroid disorders. There is some evidence to support the idea that giving vitamins with anti-oxidant features to hyperthyroidism patients can lessen the severity of their clinical symptoms. Additionally, it has been recommended that patients with autoimmune thyroid disorders undergo periodic vitamin B12 deficiency testing. B12 insufficiency in hypothyroid patients may go unnoticed due to the non-specificity of symptoms [31].

Also, the best combination was revealed in T4+Zn+Se+ Vit. B12 treatment groups as one of the best groups in the regeneration of follicular cells and reverted closely to the control group in addition to the T4+IGF-1 group. The current study's findings revealed that the thyroid gland in these groups with active follicles full of colloid fluid. The shape, arrangement, and order of follicles full of colloidal secretions were observed. Moreover, TEM ultrastructure analysis showed an active thyroid follicle with a normal nucleus, active rough endoplasmic reticulum, mitochondria, and the presence of more colloid droplets than the hypothyroid group. These results may be due to the impact of both Se and Zn, as revealed by a previous study that aimed to examine how supplementing with selenium and zinc affects the thyroid hormones in hypothyroid rat models. In comparison to the control group, giving Se and Zn at the two combined intake levels did not result in any histopathological alterations. Moreover, a combination of Se and Zn added to the diet at the studied doses improved the biochemical and histological alterations brought on by hypothyroidism. Previous studies recommended that supplementing with Se and Zn may have good effects in both healthy and diseased circumstances [19,32]. Negative correlations between goiter, thyroid tissue damage, thyroid volume, and Se status were discovered [33]. Se is essential for the proper functioning of the thyroid gland, and it is notably concentrated in this gland [34, 35]. Moreover, there is strong evidence that Vit. B12 inadequacy is more common in thyroid illness. Decreased levels of vitamin B12 were noted in patients suffering from autoimmune thyroid disease. It was discovered that both the hypothyroid and hyperthyroid states in women are linked to a reduced concentration of Vit. B12 [31].

5. Conclusion

The present investigation shows that IGF-1, Se, Zn, and Vit. B12 has the potency appropriate for the treatment of hypothyroidism which were tested both alone and in combination with T4. The treatment employed could lead to an improvement in the thyroid gland's ultrastructure, particularly in both T4+IGF-1 and T4+Zn+Se+Vit. B12 combination therapies result in the best improvement of cytological and ultrastructural changes produced by methimazole, among other treatment strategies. Furthermore, these combination treatments could cause improvement in TSH, T3, and T4 hormone levels in hypothyroidism-treated groups with fewer side effects.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

Ethics Statements

All institutional and national guidelines for the care and use of laboratory animals were followed. The procedures have received approval from the Institutional Animal Care and Use Committee (IACUC) of Ilam University according to the administrative order No. 220466 in 15/2/2021.

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