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Article

Effect of Medical Therapy on The Osteoprotegerin Levels in Patients with Hypothyroidism

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Abstract: Hypothyroidism is a clinical condition that needs primary care. It is increased with age and is more prevalent in females. Biochemical testing is used to identify hypothyroidism. Overt primary hypothyroidism is characterized by blood levels of thyroid-stimulating hormone (TSH) that are higher than normal and thyroxine levels that are lower than normal. The purpose of this research was to measure osteoprotegerin in hypothyroid individuals and look into how levothyroxine affected these patients' osteoprotegerin levels. For this purpose, 180 subjects (40 control groups and 70 patients with hypothyroidism underwent treatment, for 3 months) were selected for the study. The study materials were registered from the laboratories in Kirkuk city. The results are summarized in the following points: Osteoprotegerin Levels was highly significantly increased (0.0192) in hypothyroid patients compared to the control group, according to before and after treatment. Serum Level of Human TPO (Thyroid Peroxidase) in Women was significantly difference (0.0001significant) in hypothyroid patients compared to the control group, according to before and after treatment. The parameters of Thyroid hormones TSH, T3 and T4 were highly significantly increased ($P < 0.001$) in the serum of hypothyroid patients compared to the control group, according to before and after treatment. Osteoprotegerin was elevated significantly in hypothyroidism patients, it may be involved in the pathophysiology of hypothyroidism.

Keywords: Osteoprotegerin, hypothyroidism, Thyroid hormones, medical therapy

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1. Introduction

Hypothyroidism, or underactive thyroid, occurs when the thyroid gland fails to produce enough thyroid hormone, affecting various body systems. Caused by autoimmune illness, thyroid removal surgery, or radiation therapy, it results in high TSH and low T4 levels. Globally, it affects 4 to 5% of the population, with subclinical hypothyroidism occurring around 4-15%. Pregnancy is a risk factor, accounting for 1.5%-4% of the prevalence rate [1], [2].

Primary hypothyroidism is more common than central hypothyroidism, which is caused by an iodine deficiency. The most prevalent cause of hypothyroidism in locations with enough iodine is Hashimoto's thyroiditis, an autoimmune condition marked by autoantibodies against certain antigens and thyroid gland infiltration [3].

Thyroid stimulating hormone (TSH), which induces the thyroid to create 80% thyroxine and 20% L-triiodothyronine, leads the hypothalamus to release synthetic thyroxine, also known as levothyroxine (T4) [4]. Levothyroxine is a treatment for hypothyroidism, goiter, and thyroid cancer. It lowers thyroid-stimulating hormone (TSH) and is used as interventional therapy. However, some individuals with hypothyroidism may experience

ongoing symptoms despite normal TSH levels, requiring further laboratory and clinical evaluation [5], [6]. Levothyroxine is used to treat subclinical hypothyroidism, which is characterized by elevated TSH levels and normal-range T4 levels without symptoms. It is advised for patients with initial TSH values above 10 mIU/L, high thyroid peroxidase antibody titers, hypothyroidism symptoms, and pregnant women in order to avoid the development of hypothyroidism. The daily oral dose is 1 μg/kg [7], [8].

Osteoprotegerin (OPG) is a soluble glycoprotein with seven functional domains, including cysteine-rich N-terminal domains, death domains, and a cysteine-binding C-terminal domain. Dimerisation of OPG is crucial for RANK-RANKL inhibition, as it increases its affinity for RANKL [9], [10]. OPG regulates bone turnover through the OPG/RANK/RANKL system, with osteoblasts forming bones and osteoclasts resorbing them. RANKL, expressed by osteoblasts, binds to RANK on osteoclast surfaces [11].

High thyroid hormones cause bone turnover, primarily affecting osteoblasts and osteoclasts. Overt thyrotoxicosis reduces bone mineral density, but suppressed thyrotropin (TSH) plays a role in maintaining bone mass independently of thyroid hormones [12]. TSH receptors are linked to high bone turnover osteoporosis, suggesting that low TSH levels may contribute to skeletal loss in hyperthyroidism, not solely due to high thyroid hormones [13]. Patients with hypothyroidism may have more bone mass prior to thyroxine replacement because of altered T3, which is thought to result in a reduction in bone turnover. When treating hyperthyroidism or hypothyroidism, patients' plasma OPG levels fluctuate, showing higher baseline levels initially and lowering as therapy is administered [14].

Osteoprotegerin inhibits bone resorption and regulates vasculature, while overt and subclinical hypothyroidism increase cardiovascular disease risk [15]. A study found that plasma OPG levels increase and decrease with levothyroxine therapy in hypothyroid patients, with changes in OPG levels correlated with baseline von Willebrand factor, suggesting elevated OPG levels may increase vascular disease risk [16].

This study sought to measure osteoprotegerin levels in hypothyroid individuals and examine the impact of levothyroxine on osteoprotegerin levels in hypothyroid people.

2. Materials and Methods

2.1. Subjects and study design

This study has investigated 180 patients with hypothyroidism (70 patients before treatments, 70 patients after treatments and 40 controls healthy subjects), Seventy patients newly diagnosed hypothyroidism, underwent treatment, for 3 months and complete the follow up study their ages between (15-54) years. The patients were referred to two main facilities, Kirkuk city in Azadi hospital, and Kirkuk general hospital from November 2023 to December 2023.

2.2. Collection of samples

About 5 ml venous blood was collected from each case (before taken levothyroxine and 5 ml venous blood is drawn again after treatment of levothyroxine) by using a sterile disposable syringe then unloaded into gel tubes and allowed to clot at room temperature for 20 minutes. All samples were centrifuged at 3000 rpm for 10 minutes; sera removed and divided into four Eppendorf tubes 500 μl for each sample, then stored at - 20°C until analysis. Serum zinc-α2-glycoprotein levels, Osteoprotegerin and Human TPO (Thyroid Peroxidase) they were measured using enzyme-linked immune sorbent assay (ELISA) kits and thyroid function test (TSH, T3 and T4) by MINI VIDAS.

2.3. Statistical analysis

Microsoft EXCEL 2019 and SPSS 22 were used for data entry and analysis. Descriptive statistics were presented as frequencies and were applied to explain the characteristics of participants. The comparison between the study groups was done by t-test and Chi-Square test. A P-value of less than 0.05 will be considered statistically significant.

2.4. Ethical approval

This study was conducted based on the ethical standards stipulated in the Declaration of Helsinki. Before taking the sample, the patient's informed written and verbal agreement was obtained, after the review and approval of the study protocol and subject's information by the local ethics committee.

3. Results

Serum level of osteoprotegerin in women with hypothyroidism before and after treatment. As shown in Table 1, the mean of the serum level of osteoprotegerin in hypothyroidism women before and after treatment comparing with the control group (3.15 ± 0.28, 2.53 ± 0.28 and 2.4 ±0.26). The result was significant (0.0192).

Table 1. Comparison between hypothyroidism and healthy women regarding the mean ± SD of Osteoprotegerin Levels before and after treatment

Study groups hypothyroidism	**n**	**Osteoprotegerin (ng/mL) Mean ± SD**	**P. value**
Before treatment	70	3.15 ± 0.28	0.0192 significant
After treatment	70	2.53 ± 0.28	
Control group	40	2.42 ± 0.26	

As shown in Table 2, the mean of the serum level of Human TPO (Thyroid Peroxidase) in hypothyroidism before and after treatment comparing with the control group (6171.2 ± 506.85 versus 2966 ± 1800.9 and 2829.47 ± 279.5 pg/mL). The result was significant (P< 0.0001 significant).

Table 2. Serum Level of Human TPO (Thyroid Peroxidase) in women with hypothyroidism before and after treatment

Study groups hypothyroidism	**n**	**TPO (pg/mL) Mean ± SD**	**T. test**	**P. value**
Before treatment	70	6171.2 ± 506.85	0.19	*P< 0.0001* significant
After treatment	70	2966 ± 1800.9		
Control group	40	2829.47 ± 279.5		

The overall aim of this study was to compare the effect of levothyroxine on reducing the level of anti TPO Ab in autoimmune hypothyroid patients. According to the results of this study, before starting treatment mean ± SD (6171.2 ± 506.85), (2966 ± 1800.9) of the treatment group and (2829.47 ± 279.5) of the control group had lower levels of (Thyroid Peroxidase).

As shown in Table 3, the mean of the serum level of Thyroid Hormones in hypothyroidism before and after treatment comparing with the control group. The results of the study showed an increase in the levels of the TSH hormone in women with hypothyroidism before taking treatment compared with the results of the hormone levels after taking treatment and comparing with the control groups (32.78 ± 8.83 versus 4.26 ± 1.77 and 4.48 ± 1.68 ng/ml) respectively. The result was significant (P< 0.0001significant). While T_3 and T_4 hormone levels decreased in women with hypothyroidism before taking treatment compared with the results of the hormone levels after taking treatment and comparing with the control groups (1.49 ± 0.65 versus 6.17 ± 1.40 and 5.65 ± 2.04 ng/ml) respectively, the mean of the serum level T_4 of Thyroid Hormones in hypothyroidism before and after treatment comparing with the control group (6.85 ± 1.72 versus 14.83 ± 4.21 and 14.86 ± 3.11 ng/ml) respectively.

Table 3. Serum Level of thyroid tests in women with hypothyroidism before and after treatment

Study groups **Hypothyroidism** **140**	**Control group** **40**	**Before treatment** **Mean ± SD** **70**	**After treatment** **Mean ± SD** **70**	**P. value**
TSH (ng/ml)	4.48 ± 1.68	32.78 ± 8.83	4.26 ± 1.77	
T3 (ng/ml)	5.65 ± 2.04	1.49 ± 0.65	6.17 ± 1.40	*P< 0.001*
T4 (ng/ml)	14.86 ± 3.11	6.85 ± 1.72	14.83 ± 4.21	

Table 3 demonstrates that before levothyroxine therapy, the TSH (32.78 ± 8.83 ng/ml) values in the patients were higher than in the control participants, indicating hypothyroidism, and those cases were included in the research.

4. Discussion

In the current investigation, we discovered a significant difference (0.0192) in the basal OPG values of hypothyroid patients and healthy volunteers both before and after levothyroxine was consumed. Few research have examined OPG in thyroid dysfunctions in the literature, and the findings have been mixed. There have, as far as we are aware, been clinical investigations looking at OPG levels in hypothyroid individuals, and the results showed that OPG was greater than in controls. These investigations revealed that a possible connection between hypothyroidism and the decline in bone resorption might be OPG's role as an inhibitor of osteoclastogenesis [17].

The current study varies from earlier research by Özdemir et al., which found that there was no discernible difference (0.844) between OPG levels in hypothyroid patients before and after levothyroxine use in comparison to control groups [18].

Büchi, Annina Elisabeth, et al. reported similar findings in their prior study, stating that the results are encouraging considering the large number of patients whose levothyroxine-treated hypothyroidism adversely affects bone health. It is advised that doctors who treat women with hypothyroidism administer the levothyroxine needed to produce a clinical response and maintain TSH within the reference range until long-term safety is established [19].

According to Jayash et al. [20], there was an increase in bone mass following OPG therapy at doses of 10 mg/kg/day for seven days and 24 mg/kg/day for fourteen days. Moreover, hypothyroid individuals' ovariectomy-induced bone loss has been totally avoided when OPG therapy is administered at a dose of 5 mg/kg/day for 14 days. The outcome demonstrated that a decrease in the number of osteoclasts was correlated with increases in bone mineral density and volume. The evidence indicated that the injection of OPG, which lowers the quantity of calcium in the blood of healthy individuals, suppresses

bone resorption by inhibiting osteoclastogenesis, particularly the maturation of osteoclasts [20], [21].

To summarize the major findings, we discovered that the outcome was significant (P<0.0001significant) in women with or without thyroid autoantibodies in the current prospective research. Based on our results, it is recommended that thyroid autoimmune illness be taken into account while investigating female hypothyroidism problem.

Nonetheless, given that the general population's prevalence of TPOAb was not different from that of the research population, it is possible that thyroid autoimmunity may not directly cause infertility, since its prevalence would have been higher in any other case [22], [23]. According to Negro et al. (2015), there is a non-significantly larger proportion of TPOAb women among women with hypothyroidism disorder, where raised TSH levels are a powerful preventive measure against hypothyroidism disease [24].

One of the recognized causes of the autoimmune thyroid disease that leads to hypothyroidism is TPO-Ab; there may be a positive correlation between reduced thyroid function and TPO-Ab titer levels. Furthermore, a prior cross-sectional investigation found that women with hypothyroidism had considerably greater blood levels of human TPO than normal control participants prior to levothyroxine therapy [25]. These findings suggest that decreased thyroid function levels rather than TPO-Ab levels may be linked to hypothyroidism condition [26]. In the current investigation, however, we discovered a statistically significant positive correlation between TPO-Ab titer and hypothyroidism disease in women before to levothyroxine medication as opposed to women following levothyroxine treatment and control groups.

Conversely, Kachouei et al.'s study found that while there was no discernible difference in the control group's thyroid hormone levels before and after treatment, the anti-TMP receiving group's levels did show a substantial decline. However, FT3 and FT4 levels dropped to the same point in both groups. Stated differently, it may be inferred that there has been a notable decrease in the levels of anti-thyroid hormone antibodies following a rise in serum Thyroid Peroxidase levels [27].

While research by Negro et al. and van Zuuren et al. similarly showed that levothyroxine medication reduced the anti-TPO titer, [28], [29] indicated in studies It has been demonstrated that LT4 therapy is beneficial for autoimmune thyroiditis. According to a research by Onal et al., women with hypothyroidism disease may have significantly lower levels of anti-TPO before receiving levothyroxine medication as opposed to thereafter, as well as in control groups [30]. The current study differs from that of Anastasilakis et al. in that it found no discernible impact of medication on the level of human thyroid peroxidase (TPO) in individuals with autoimmune hypothyroidism [31].

Based on the findings of this study and others, it is generally concluded that treating hypothyroidism with levothyroxine may help lower levels of human TPO (thyroid peroxidase). However, the current study's limitations, such as its small sample size and short follow-up period, mean that the evidence for the effectiveness of treating hypothyroidism with levothyroxine is still insufficient [27]. Therefore, more research is needed to determine the amount of human TPO with a bigger sample size, a longer follow-up time, and other variables.

Because the action of estrogen is higher in reproductive age compared to perimenopausal or menopausal age, hypothyroidism was detected in females in our study. Estradiol competes with T3, T4, and other hormones for the binding receptor proteins, which is why it plays a role in the pathophysiology of hypothyroidism. As a result, there may be less T3, T4, and other hormone activity in the blood, which might cause hypothyroidism. Other investigations, including Shantha et al., have reported comparable findings [32].

The pace at which the body utilizes energy, or metabolism, is largely controlled by thyroid hormones [33]. They do this by promoting a variety of metabolic processes in most tissues, raising the basal metabolic rate, and boosting body heat output [34] shows that

obesity is frequently associated with thyroid function issues, with a high incidence of hypothyroidism [35].

The findings indicate that, in comparison to the control group, there was a significant increase in TSH concentrations and a significant decrease in thyroid hormone (T3, T4) concentrations in obese and overweight individuals. These findings are consistent with research by Nils Knudsen et al. [36] and M. Bastemir et al. [37] that found that even slightly elevated serum TSH levels were associated with an increased risk of obesity [38]. Due to the thyroid gland's significant biological influence on development, reproduction, and metabolic control, among other bodily functions.

The increased requirement for ATP brought on by lower ATP synthesis efficiency and higher activity in most cells is the source of thyroid hormone-induced thermogenesis. Therefore, a delay and lack of metabolic activity are characteristics of hypothyroidism patients, and this frequently results in a rise in the rate of BMI [39]. Prior studies have connected hypothyroidism to elevated levels of oxidative stress in general; they also found that elevated levels of oxidative stress may be detrimental to thyroid follicular cells, which release T3 and T4, reducing their blood levels and elevating TSH [40].

Additionally, the present findings concurred with research cited by Thenmalar. In their research on the diagnosis and clinical manifestations of hypothyroidism in the elderly and age-related conditions, Vadiveloo et al. (2013) [41] noted that an increase in blood TSH levels is frequently observed in older people. Together with their elevated TSH levels, they also had disordered T4 and lowered T3 readings. However, a different study revealed that as age advanced, serum TSH decreased and T4 increased in hypothyroidism [42].

Comparatively, similar findings were found in another study conducted in India by Dhok et al. [43], with hypothyroidism being the most prevalent disease among individuals with thyroid disorders in the current study.

The frequency of thyroid problems in older women was significantly positively correlated. In a different study conducted in India, Negro et al. found that those over 30 had a noticeably greater frequency of thyroid illness [44].

The frequency of thyroid disorders and family history of thyroid illness were shown to be significantly correlated. Gregory concurred, reporting that there is a substantial family history of thyroid illness in women [45]. Another research conducted in India produced the same outcomes [43]. On the other hand, research by Rajput et al. and Mahadik et al. showed no connection between family history and thyroid illness during pregnancy [45], [46].

5. Conclusion

Patients with hypothyroidism had considerably higher levels of osteoprotegerin. Osteoprotegerin may therefore have a role in the pathogenesis of hypothyroidism. When comparing hypothyroidism to the control, there was a substantial rise in the amount of human TPO. Patients with hypothyroidism who were female showed a strong positive connection with the thyroid hormones TSH, T3, and T4.

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