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RESEARCH ARTICLE

Effects of Metformin Therapy on Thyroid Volume and Functions in Patients with Newly Diagnosed Type 2 Diabetes Mellitus: A Single-center Prospective Study

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Abstract: ***Objective*:** Patients with impaired glucose metabolism have increased thyroid volume and a higher prevalence of nodules. Yet, some studies show that there is an improvement in these thyroid parameters after diabetes treatment. Our observational study aimed to reveal the effect of treatment on thyroid function, thyroid volume, and the presence of nodules in newly diagnosed type 2 diabetes mellitus (T2DM) patients who were started on metformin treatment.

***Methods*:** Euthyroid and subclinically hypothyroid patients with a serum TSH level of <10 mU/L, who were newly diagnosed with T2DM and started on metformin as an antidiabetic treatment and not used any thyroid medication previously, were included in our study. Patients' characteristics were recorded. Baseline and 6th-month serum thyroid function tests were scheduled. Baseline and 6th-month thyroid gland characteristics were examined by thyroid ultrasonography.

***Results*:** A total of 101 (37 males, 64 females) newly diagnosed T2DM patients with euthyroid (n=95) or subclinical hypothyroidism (n=6) were included in the study. The mean age of the patients was 53.02 ± 11.9 years, and the mean BMI was 29.60 ± 3.9 kg/m^2. Fifty-two (52%) patients were classified as obese. Body weight, BMI, serum TSH, ALT, Anti-TPO levels, and thyroid volume decreased significantly in the 6th-month compared to baseline values (p = 0.000; p = 0.000; p = 0.011; p = 0.022; p = 0.000, respectively). Serum anti-Tg, fT4, fT3 levels, and thyroid nodule count did not change significantly. A high agreement was found between the baseline and 6th-month nodule counts (gamma= 0.886; p < 0.001) and the presence of multi-nodularity in the thyroid (gamma= 0.941; p < 0.001), but no significant change was observed. Anti-TPO levels showed a significant decrease in both with and without obesity groups at the end of 6 months (p = 0.003, p = 0.009, respectively). Serum TSH level decreased significantly only in non-obese subjects (p = 0.004), and thyroid volume decreased significantly only in obese subjects (p = 0.000).

***Conclusion*:** Our results suggest that metformin treatment significantly reduces body weight, BMI, thyroid volume, and serum TSH, ALT, and Anti-TPO levels in patients with newly diagnosed T2DM. Moreover, serum TSH levels showed a significant decrease in non-obese subjects, while thyroid volume showed a significant decrease in obese subjects.

Keywords: Type 2 diabetes mellitus, metformin, TSH, thyroid volume, thyroid nodule, obesity.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a heterogeneous disease, and its etiology is likely to involve multiple primary genetic and environmental factors leading to relative insulin deficiency. These patients are generally adults with obesity and may present with a progressive decrease in beta cell capacity and hyperglycemia that worsens over time [1, 2].

It is well documented that most of the hormones in the body, due to their antagonistic effects against insulin, lead to an increase in hepatic glucose output and a decrease in its use in peripheral tissues (particularly in muscle and adipose tissue). Hence, some endocrinological diseases (*e.g.*, acromegaly, Cushing's syndrome, hyperthyroidism, hypothyroidism, polycystic ovary syndrome (PCOS), hypogonadism and other hormonally active neuroendocrine tumors) lead to fluctuations in insulin sensitivity [3]. The association of insulin resistance (IR) with thyroid function and morphologic abnormalities has been an interesting area of research in recent years, and increased insulin levels due to IR have led to in-

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creased levels of insulin-like growth factor-1 (IGF-1). Moreover, IGF-1 plays a significant role in the hypertrophy and progression of a variety of cell types, including thyroid cells. Experimental evidence suggests that both insulin and IGF-1 modulate the regulation of thyroid gene expression and that high levels of insulin and/or glucose can lead to the proliferation of thyroid cells by affecting cellular energy metabolism. The progression of the thyroid cell cycle depends on the combined activity of thyroid stimulating hormone (TSH) and insulin/IGF-1, all of which function as co-mitogenic factors. Insulin also increases the mitogenic activity of growth factors. Although the proliferative properties of insulin and IGF-1 are well-known, the impact of these parameters on total thyroid volume is not clear. Nonetheless, some studies have revealed that increased thyroid volume is associated with impaired glucose metabolism [4-6]. Individuals with hyperinsulinemia had a larger thyroid gland and a higher prevalence of thyroid nodules and cancers [7, 8]. TSH and the insulin/IGF1 axis play a key role in nodule formation. The classical Thyroid Stimulating Hormone Receptor (TSHR), mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR) pathways act to modulate the regulation of thyroid gene expression and other processes that may be involved in thyrocyte proliferation, differentiation, and malignant transformation [9, 10].

Thyroid dysfunction is also more common in diabetic patients than in the general population [11, 12]. In a region with mild to moderate iodine deficiency, mean serum TSH level, thyroid volume, and nodule prevalence have been shown to be significantly increased in patients with prediabetes and T2DM compared to the control group [6]. In the study, significant increases were observed in the median thyroid volume of adult patients with both T2DM and T1DM who did not have overt thyroid disease but were referred to the study due to poor glycemic control or diabetic complications, compared to the control group and thyroid nodules and goiter were more common in the population with T2DM [4, 5]. Both a systematic review and a case-control study showed a statistically significant increased risk of thyroid cancer due to high thyroid volume in individuals with diabetes compared to non-diabetics [10, 11].

Metformin is the most commonly prescribed antidiabetic drug in T2DM. At the cellular level, it acts by indirectly activating AMP-activated protein kinase (AMPK) and partially inhibiting glycerol phosphate dehydrogenase (m-GDP). It has a suppressive effect on lipid and cholesterol biosynthesis through transient inhibition of mitochondrial respiratory chain complex 1 [13]. Metformin is a hydrophilic drug that exists mainly in a positively charged form under physiological conditions. Intestinal absorption of metformin is mediated primarily by the plasma membrane monoamine transporter (PMAT, SLC29A4 gene), which is localized on the luminal side of enterocytes. Organic cation transporter 1 (OCT1, SLC22A1 gene) is expressed on the basolateral membrane of enterocytes and may be responsible for the transport of metformin into the interstitial fluid. The primary mediator of hepatic metformin uptake is OCT1 and possibly OCT3 (SLC22A3 gene) expressed in the basolateral membrane of hepatocytes [14].

Some studies conducted with metformin in recent years have demonstrated that it has antitumor, anti-aging, cardiovascular protection, and neuroprotective properties, which occur through its positive effect on AMPK [15]. Experimental studies have demonstrated that metformin has an inhibitory effect on the growth of human thyroid cells. Thus, it has been hypothesized that the association between T2DM and goiter, if present, could be modulated by metformin use [16]. In a study using data from a population-based German cohort study on T2DM, metformin was shown to have a goiter-inhibiting effect [13]. Several *in vitro* and *in vivo* studies have shown that metformin can inhibit the growth of thyroid cells and different types of thyroid cancer cells by affecting the insulin/IGF1 and mTOR signaling pathways [17, 18]. Furthermore, metformin treatment caused a decrease in serum TSH levels in diabetic patients, possibly by enhancing the effects of thyroid hormones in the pituitary and activating AMPK [9]. Based on this evidence, metformin appears to be a promising therapeutic tool in patients with thyroid disease.

It has also been shown that metformin decreases serum TSH levels in women with subclinical hypothyroidism (SH) and diabetes/ impaired fasting glucose (IFG) but does not affect thyroid hormone and prolactin levels in men and women. The results suggest that gender may determine the effect of metformin on hypothalamic-pituitary-thyroid axis activity [19]. Additionally, several studies have shown that metformin reduces serum TSH levels in patients with overt hypothyroidism. It has also been reported that metformin may play a protective role against the growth of thyroid nodules in patients without IR and may sensitize TSHR to TSH by affecting the IRS1 phosphorylation pattern [20]. However, the results show that metformin has a negligible effect on hypothalamic-pituitary-thyroid axis activity in patients with T2DM with subclinical hyperthyroidism than in patients with untreated subclinical hyperthyroidism [21, 22].

Our study aimed to show the effect of metformin treatment on thyroid function, thyroid volume, and the presence of nodules in newly diagnosed T2DM patients who were started on treatment in line with the data mentioned above.

2. MATERIALS AND METHODS

2.1. Study Design and Patient Selection

In this prospective study, 101 adults (37 males, 64 females) who were started on metformin for newly diagnosed T2DM patients between March 2018 and October 2018 at Çukurova University Balcalı Hospital Internal Medicine outpatient clinic were included as the case group. All patients had been diagnosed with T2DM according to American Diabetes Association 2018 criteria [23]. In our study, we excluded patients who exhibited previously known thyroid dysfunction. The study patients were selected from patients whose serum TSH level is <10 mU/L [with euthyroid and subclinical hypothyroidism) and who have not used any thyroid medication. Demographic characteristics, body mass index (BMI), comorbidities, medications used, and starting dose of metformin were recorded. Exclusion criteria were defined as the use of drugs that may affect serum TSH levels, such as oral contraceptives, the presence of chronic kidney disease, chronic liver disease, heart failure, sepsis, pregnancy, and T1DM [24, 25]. During the follow-up period,

follow-up was continued in a small number of patients who needed other antidiabetic treatments in addition to metformin treatment. Consent has been obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used. The study was approved by the Çukurova University Ethics Committee (date: April 13, 2018, meeting number: 76, decision no: 101).

2.2. Measurement of Patients' Laboratory Parameters

In order to examine the baseline and 6th-month fasting plasma glucose (FPG), HbA1c, complete blood count, blood urea nitrogen (BUN), creatinine, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), TSH, fT4, fT3, Anti-TPO, and Anti-Tg levels, venous blood samples were collected from patients in the morning after 8 hours of fasting at night.

Insulin resistance was calculated using the Homeostatic Model Assessment - Insulin Resistance (HOMA-IR) formula [26]:

Patients with HOMA-IR= Fasting Glucose (mg/dL) X Fasting Insulin (uIU/mL) / 405 ≥2.5 were considered insulin resistance positive (HOMA-IR [+]).

Using the serum obtained after centrifugation of venous blood taken into a gel biochemistry tube, fT_3, fT_4, TSH, Anti-TPO, and Anti-Tg levels were measured by chemiluminescence technique *via* Beckman Coulter Unicel DXI800 device. In addition, glucose, BUN, creatinine, AST, and ALT levels were measured in serum samples of all patients with a Beckman Coulter AU5800 device. Glucose was measured using the hexokinase method, while BUN was measured using the urease, a colorimetric method. Creatinine was measured using the kinetic alkaline picrate method, ALT by UV without the P5P method, and AST by U without the P5P method.

Hemoglobin and White blood cells (WBC) were measured with a Beckman Coulter Unicel DXH800 device. Hemoglobin was measured using the photometric method, and WBC was measured using the electrical impedance method. Hemoglobin A1c was measured using the HPLC-Boronate Affinity method using Premier HB9210.

In accordance with hospital biochemistry laboratory criteria, TSH: 0.35-4.2 mU/L, fT3: 2.3-4.2 pg/ml (0.53-6.45 pmol/L), and fT4: 0.89-1.8 ng/dl (11.45-23.16 pmol/L) were considered normal serum values. Patients with serum TSH of >4.20 mU/L and fT3 and fT4 values within normal ranges were considered to have subclinical hypothyroidism [27, 28].

2.3. Ultrasonographic Evaluation of the Thyroid Gland in Patients

Real-time US of the thyroid gland is usually performed with high-resolution linear array transducers ranging from 7.5 to 10 MHz. High-resolution thyroid US is the most sensitive method for the detection of nodules [29, 30].

The baseline and 6th-month thyroid volume, the presence and number of nodules, the largest nodule diameter (mm), and the presence of multi-nodularity of our patients were examined *via* the GE Healthcare LOGIQ P6 Ultrasonography Device from 2013. The same physician performed examinations to avoid practitioner-dependent variations. During the procedure, the patients were placed in the supine position with their necks in slight extension. Thyroid volume was calculated with the formula "depth (cm) x width (cm) x length (cm) x π/6" [31]. For thyroid nodule size (mm), the longest measured diameter of the nodule was considered. Detection of two or more nodules on the thyroid US was considered multinodular [32].

2.4. Statistical Analysis

The SPSS 20.0 package program was used for the statistical analysis of the data. Categorical measurements were expressed as numbers and percentages, while continuous measurements were summarized as mean and standard deviation (median and minimum-maximum where necessary). Gamma fit statistic was used to determine whether categorical measurements changed during the follow-up period. In the determination of whether the continuous measurements changed during the follow-up period, the dependent samples t-test was used if the assumptions were met, and the Wilcoxon signed-rank test was used if the assumptions were not met. Changes in continuous measurements over time were obtained by subtracting the 6th-month measurement from the baseline measurement. Independent samples t-test was used to compare these differences between the groups. The results were considered significant at $p < 0.05$ in all tests.

3. RESULTS

3.1. Demographic and Clinical Characteristics of the Patients

A total of 101 newly diagnosed T2DM patients with euthyroidism (n=95) or SH (n=6) were included in the study (37 males (36.6%), 64 females (63.4%)). The clinical characteristics of the patients at the beginning of the study are presented in Table **1**.

The mean BMI of the patients was 29.60 ± 3.9 kg/m^2. Fifty-two patients (52%) had obesity (BMI: 30.00-34.99 kg/m^2 in 41 patients, BMI: 35.00-39.99 kg/m^2 in 8 patients, and BMI >40.00 kg/m^2 in 3 patients). The baseline HOMA-IR score was ≥2.5 in 79.4% of the patients. Thirty-six patients (35.6%) had no history of comorbidity. However, the most common comorbidities were hypertension (41 patients, 40.6%) and hyperlipidemia (15 patients). Antihypertensive medication use (41 patients, 40.6%) was the most common additional medication (usually amlodipine, doxazosin, or nebivolol). The metformin dose started at the beginning of the study, and additional antidiabetics in the follow-up period are shown in Table **2**. The majority of patients (78%) used 2000 mg of metformin daily, and 73% of patients used metformin as the only antidiabetic and did not require any additional antidiabetic.

3.2. Changes in Biochemical Parameters and Thyroid Function Tests of Patients Before and After Treatment

The white blood cell count (WBC), Hb, creatinine, AST, fT4, fT3, and Anti-Tg did not change significantly within 6 months of follow-up of the patients. The mean baseline body weight of the patients was 83.57 ± 10.61 kg, while the month-6 value showed a significant decrease (81.77 ± 9.87) compared to the baseline value (p = 0.000). In addition to

Table 1. Baseline clinical characteristics of the patients (mean ± SD)*.

Variables **	Euthyroid (n=95)	Subclinical Hypothyroidism (n=6)	Total (n=101)
Age (years)	53.39±11.63	47.17±14.87	53.02±11.9
Height (m)	1.68±0.07	1.65±0.10	1.68±0.08
Weight (kg)	83.58±10.05	84.00±14.24	83.57±10.61
BMI (kg/m^2)	29.52±3.81	30.78±5.23	29.60±3.9
FPG (mmol/ L)	8.1±2.9	10.2±3.0	8.0±2.8
HbA1c (mmol/mol)	56±4	70±17	57±9
Insulin (pmol/ L)	92±55	125.5±121.7	94.7±62

Note: *Mean ± standard deviation; ** BMI: Body Mass Index, FPG: Fasting Plasma Glucose, HbA1c: Hemoglobin A1C.

Table 2. The dose of metformin started at the beginning of the study and additional antidiabetics.

Metformin Dose	n; %	Additional Antidiabetics*	n; %
500 mg/day	3; 3%	No	73; 73%
1000 mg/day	14; 14%	Thiazolidinedione	3; 3%
2000 mg/day	78; 78%	DPP4 inhibitor	9; 9%
850 mg/day	4; 4%	SU or Glinid	2; 2%
1700 mg/day	2; 2%	SGLT2 inhibitor	1; 1%
-	-	Combined OAD	7; 7%
-	-	Baseline insulin	2; 2%
-	-	Premix insulin	1; 1%
-	-	Intensive insulin	2; 2%

Note: *DPP4: Dipeptidyl peptidase 4**; SU:** Sulfonylurea; **SGLT2:** Sodium glucose cotransporter 2; **OAD:** Oral antidiabetic.

body weight, BMI, FPG, HbA1c, ALT, TSH, Anti-TPO levels, and thyroid volume showed a significant decrease. The changes in the clinical findings of the patients before metformin treatment and at the 6th-month are shown in Table **3**.

While the baseline serum TSH levels of the patients were 1.89 ± 1.15 mU/L, the mean serum TSH level after 6 months of follow-up was 1.67 ± 1.21 mU/L, and this decrease in serum TSH level was significant (p = 0.011). The change in serum TSH level during the study follow-up is shown in Fig. (**1**).

Since the numbers of patients with euthyroidism and SH are very different from each other, and the number of patients with SH is very small, the effectiveness of metformin treatment on thyroid hormones, thyroid volume and nodules in these two patient groups could not be compared with each other.

3.3. Ultrasonography Findings of Patients at the Beginning and End of the Study

It was aimed to evaluate all patients with thyroid US at baseline and month 6, but only 66 patients could be evaluated with US at baseline and month 6 due to patients lost to follow-up. The maximum nodule diameter did not change significantly during the 6 months of follow-up (p = 0.927). While the mean thyroid volume of the patients was 13.60 ± 8.01 mL, it was 10.25 ± 6.63 mL in the 6th-month, and this decrease was statistically significant (p = 0.000). The mean decrease in thyroid volume observed throughout the study is shown in Fig. (**2**).

A total of 35 patients who voluntarily discontinued follow-up or could not be reached during the study could not undergo thyroid US at 6 months. Therefore, only 66 of the 101 patients included in the study at baseline were able to undergo thyroid US both at baseline and at 6 months. Thirty-five of these 66 patients had no thyroid nodules at the start of the study. Among patients with nodules at baseline (n=31), 11 patients had 1 nodule, 4 patients had 2 nodules, and 16 patients had 3 or more nodules. Following treatment, 1 nodule in 4 patients and 2 nodules in 1 patient were free of nodules after 6 months. While 5 patients had no nodules before treatment, nodule formation was observed in these patients at the 6th-month follow-up (1 of these patients developed 2 nodules). A high agreement was found between the baseline and 6th-month nodule counts of the patients (gamma = 0.886, $p < 0.001$). Accordingly, there was no statistically significant change in the number of nodules during the follow-up.

Table 3. Changes in pre-treatment and post-treatment clinical and laboratory findings (mean ± SD).

**Variables	Pre-treatment	Post-treatment	*P
Weight (kg)	83.57±10.61	81.77±9.87	0.000
BMI (kg/m2)	29.60±3.98	28.97±3.79	0.000
WBC (u L)	8.12±2.08	8.00±1.80	0.509
Hb (mmol/ L)	8.48±1.05	8.5±1.05	0.694
FPG (mmol/ L)	8.0±2.7	6.5±1.8	0.000
HbA1c (mmol/mol)	58±4	46±4	0.000
Creatinine (µmol/ L)	59±13.2	57.4±14.5	0.100
ALT (IU/L)	25.39±13.93	22.24±11.56	0.022
Vitamin B12 (pmol/L)	184.5±94	176±67	0.359
Total- C (mmol/ L)	5.5±1.5	5.47±1.26	0.630
LDL- C (mmol/ L)	3.6±1.3	3.48±1.13	0.367
HDL-C (mmol/ L)	1.1±0.2	1.1±0.24	0.141
Triglyceride (mmol/ L)	1.97±1.08	1.8±0.92	0.189
TSH (mU/L)	1.89±1.15	1.67±1.21	0.011
fT4 (pmol/L	11.19±4.76	10.9±1.8	0.593
fT3 (pmol/L)	5.34±0.89	5.43±0.62	0.380
Anti TPO (U/mL)	28.42±106.93	25.80±94.86	0.000
Anti Tg (U/mL)	10.41±55.25	14.61±91.51	0.086
Thyroid volume (mL)	13.60±8.02	10.25±6.63	0.000
Maximum nodule diameter (mm)	16.80±11.84	16.56±10.81	0.927

Note: *$p < 0.05$ was considered statistically significant; ** BMI: Body Mass Index**, WBC:** White blood cell**, Hb:** Hemoglobin**, FPG:** Fasting Plasma Glucose**, HbA1c:** Hemoglobin A1C**, AST:** Aspartate Aminotransferase**, ALT:** Alanine Aminotransferase**, Total-C:** Total Cholesterol, **LDL-C:** Low-Density Lipoprotein-Cholesterol**, HDL-C:** High-Density Lipoprotein-Cholesterol**, TSH:** thyroid stimulating hormone, **fT4:** free thyroxine**, fT3:** free triiodothyronine**, Anti-TPO:** anti-thyroid peroxidase antibody, **Anti-Tg:** anti-thyroglobulin antibody.

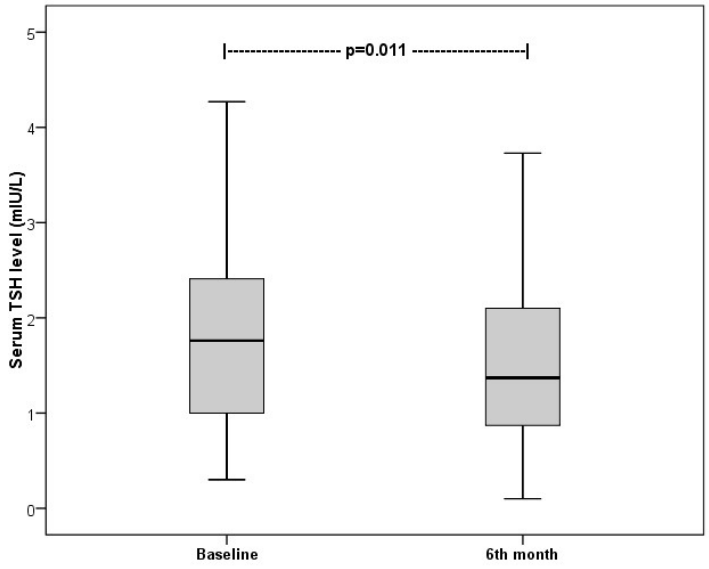


Fig. (1). The decrease in serum TSH level seen in patients in the study at baseline and after 6 months follow-up (midline = median value). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

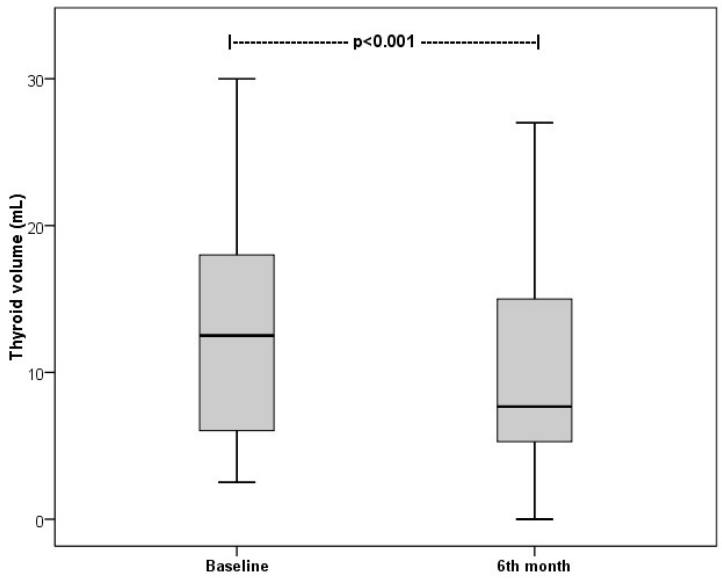


Fig. (2). The decrease in thyroid volume seen in patients in the study at baseline and after 6 months follow-up (midline = median value). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

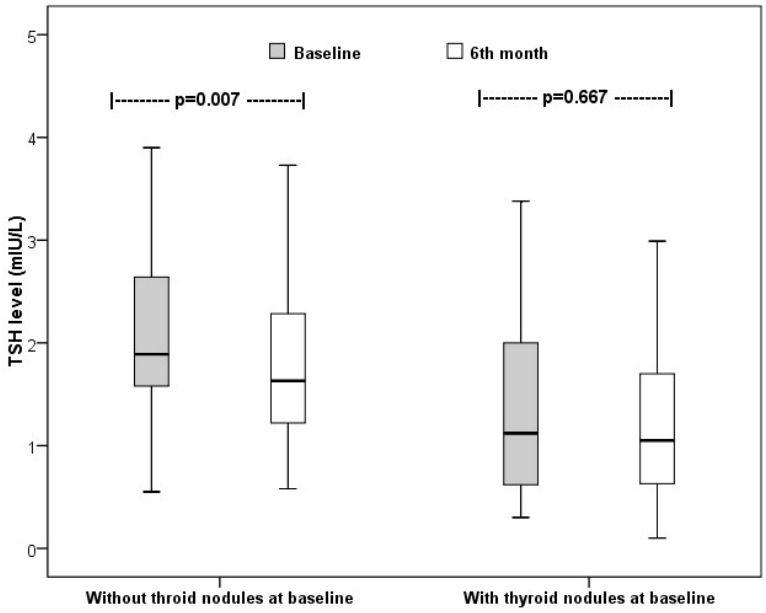


Fig. (3). Changes in serum TSH levels throughout the study in groups with and without thyroid nodules at the beginning of the study. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Compared to the groups with and without nodules before treatment, serum TSH levels decreased in both patients with and without nodules at baseline and 6th-month. Still, this decrease was statistically significant only in those without nodules (p = 0.007). Fig. (**3**) shows the change in serum TSH levels throughout the study in groups with and without thyroid nodules at the beginning of the study.

Detection of 2 or more nodules on the thyroid US was considered multinodular. While multi-nodularity was observed in 20 of 66 patients at the beginning of the study, multi-nodularity was not observed in 4 of these patients after treatment. A high agreement was found in terms of the presence of multi-nodularity at the baseline and 6th-month (gamma = 0.941, p < 0.001). Accordingly, there was no statistically significant change in the presence of multi-nodularity in the patients throughout the follow-up.

Table **4** shows the demographic and metabolic values in non-obese and obese patients. In the non-obese group, mean serum TSH levels showed a significant decrease at 6 months compared to baseline values (p = 0.004). Besides, thyroid volume decreased in all obese and non-obese patients at 6 months, but this decrease was only statistically significant in obese patients (p = 0.000). Moreover, body weight, serum ALT, and Anti TPO levels also showed statistically significant decreases in obese and non-obese subjects. It was observed that those who were obese lost numerically more

Table 4. Baseline and at the 6th Month clinical characteristics and metabolic parameters in obese and non-obese patients (mean ± SD).

Non-obese (n=49)				Obese (n=52)		
****Variables**	**Baseline**	**Month-6**	***p***	**Baseline**	**Month-6**	***p***
Weight (kg)	77.30±9.24	75.84±8.85	0.001	89.45±8.20	87.32±7.23	0.000
WBC (u L)	8.20±2.06	8.32±1.75	0.672	8.04±2.10	7.70±1.80	0.130
Hb (mmol/ L)	8.5±1.12	8.6±1.1	0.322	8.4±0.97	8.37±0.98	0.620
FPG (mmol/ L)	7.7±2.1	6.6±1.9	0.001	8.4±3.2	6.4±1.6	0.000
HbA1c (mmol/mol)	55±4	44±4	0.000	61±4	48±4	0.000
Creatinine (µmol/ L)	60±12.3	60±15	0.410	57.4±13	55.7±11.4	0.127
ALT (IU/L)	26.91±11.26	24.25±10.6	0.046	23.93±16.06	20.33±12.16	0.019
TSH (mU/L)	1.95±1.21	1.69±1.30	0.004	1.84±1.10	1.67±1.12	0.227
fT4 (pmol/L)	10.9±1.9	11.06±1.9	0.669	11.5±6.3	10.8±1.54	0.509
fT3 (pmol/L)	5.43±0.73	5.57±0.62	0.166	5.26±1.01	5.29±0.56	0.855
Anti TPO (U/mL)	39.86±129	36.20±116	0.009	18.33±83.40	16.63±71.39	0.003
Anti Tg (U/mL)	17.02±79.38	26.93±134.4	0.540	4.78±17.80	4.11±12.33	0.067
Thyroid volume (mL)	12.51±7.76	10.98±6.74	0.124	14.51±8.21	9.64±6.57	0.000
Maximum nodüle Diameter (mm)	17.83±15.26	18.42±13.20	0.858	15.85±8.05	14.85±8.19	0.752

Note: *$p < 0.05$ was considered statistically significant; **** BMI:** Body Mass Index**, WBC:** White blood cell**, Hb:** Hemoglobin**, FPG:** Fasting Plasma Glucose**, HbA1c:** Hemoglobin A1C**, AST:** Aspartate Aminotransferase**, ALT:** Alanine Aminotransferase**, TSH:** thyroid stimulating hormone, **fT4:** free thyroxine**, fT3:** free triiodothyronine**, Anti-TPO:** anti-thyroid peroxidase antibody, **Anti-Tg:** anti-thyroglobulin antibody.

weight and had a greater decrease in FPG and HbA1c compared to those who were not obese, but this difference was not statistically significant.

4. DISCUSSION

In this prospective clinical study, we planned to investigate the effect of treatment on metabolic parameters, thyroid function, thyroid volume, and the presence of nodules in euthyroidic and subclinically hypothyroidic T2DM patients admitted to our outpatient clinic, newly diagnosed and started treatment. In order to obtain a more homogeneous group and considering the previous positive data on metformin, patients diagnosed with T2DM and started on metformin were included in our study.

In our study, we found that glycemic parameters (FPG and HbA1c), as well as body weight, serum ALT, TSH, and Anti-TPO levels and thyroid volume, decreased after 6 months of follow-up with metformin treatment. In addition, serum TSH levels decreased significantly in non-obese patients after 6 months of follow-up, whereas thyroid volume decreased significantly in obese patients.

Diabetes mellitus is one of the most common public health problems, with T2DM being the most common form of diabetes worldwide, accounting for 90% of global cases. Globally, the prevalence of diabetes is similar among men and women but slightly higher among men under the age of 60 and women over the age of 60. In developing countries, the vast majority of people with diabetes are between the ages of 45 and 64 [33]. The mean age of our patients at the start of the study was 53.02 ± 11.9 years, and 63.4% of them were female.

Among our patients, hypertension was the most common comorbidity, and antihypertensive medications were the most commonly prescribed medications. In 48.5% of our patients, there was no additional drug use except for the antidiabetic drug in addition to metformin [34, 35]. In patients taking additional medications, it should always be considered whether these medications have a positive or negative effect on the efficacy of metformin. During follow-up, 27 of our patients were started on oral or parenteral antidiabetic drugs in addition to metformin treatment, and 9 of these drugs were DPP4 inhibitors. Repaglinide, an oral antidiabetic drug, has been shown to inhibit the organic OCT1 responsible for the transport of metformin into the interstitial fluid, and sitagliptin, a DPP4 inhibitor, has been shown to inhibit both OCT1- and OCT2-mediated transport of metformin. Therefore, it can be thought that these drugs may theoretically affect the intestinal and hepatic uptake of metformin [36, 37]. When other drugs were evaluated in terms of affecting the pharmacokinetics of metformin, studies showed that metformin was not affected by co-administration of dapagliflozin, hydrochlorothiazide, ipragliflozin, linagliptin, orlistat, rosiglitazone, rosuvastatin, saxagliptin, vildagliptin, and beta-blockers [38, 39].

Since the number of patients using repaglinide, DPP4 inhibitors, or other drugs, including insulin treatment, was small in our study, it is unlikely that we can obtain sufficient data on the positive or negative effects of these drugs on metformin. In addition, the effects of the same drugs on thyroid function, volume, and nodules could not be evaluated.

In studies on the use and doses of metformin therapy, it is recommended to take metformin at meals and in low doses,

especially to minimize gastrointestinal adverse effects. It is generally recommended to administer 500 mg once or twice a day, up to a maximum tolerated dose of 1000 mg twice daily, up to 2000 mg in weekly increments. More than 50% of the drug's efficacy has been observed at a dose of 1000 mg/day. Accordingly, the administration of 1000-1500 mg daily in patients who have problems at higher doses should be considered highly effective [40]. In our study, the metformin therapy dose was 2000 mg/day in 78 patients, and the patients have well tolerated this dose.

Since approximately 90% of people with T2DM are overweight or obese, obesity appears to be the most important risk factor for the development of T2DM. The primary reason for the interest in metformin today is its potential as a weight loss drug. Recent studies on obesity suggest that changes in gastrointestinal physiology and circadian rhythm by metformin have an impact not only on food intake but also on fat oxidation and the regulation of fat storage in the liver, skeletal muscle, and adipose tissue [41]. As demonstrated in long-term follow-ups in the Diabetes Prevention Program, metformin promotes permanent weight loss, primarily by reducing food intake due to its effects. Although the effect of metformin on appetite is multifaceted, its effects on hypothalamic physiology, including leptin and insulin sensitivity, have been demonstrated [42]. In our study, 52 (51.5%) of the patients had obesity when diagnosed with T2DM. Similar to the results of the studies above, a statistically significant decrease in body weight was observed in our patients after 6 months of metformin treatment. In our patients, there was no significant difference in weight loss between those with and without obesity at baseline.

In cross-sectional studies, we have shown that the prevalence of thyroid dysfunction was found to be between 16.2% and 56.5%, with a higher prevalence in women in the over-50 age group and patients with poor glycemic control. Besides, it has been shown that there is a correlation between the duration of T2DM and an increased risk of thyroid dysfunction, although not statistically significant [43]. In a meta-analysis including studies conducted for 34 years investigating the relationship between T2DM and SH, it was reported that the prevalence of SH in T2DM patients was 10.2%, and there was a 1.93-fold increase in the risk of SH in T2DM patients. Furthermore, the presence of SH may affect the development of diabetic complications in T2DM patients [44]. At the beginning of our study, 6 patients (6%) had SH, 10 patients (10%) were anti-TPO positive, and thyroid dysfunction was observed less than expected, considering that patients over 50 years of age and female patients were in the majority. The low rate of thyroid dysfunction might be attributed to the fact that patients with relatively good glycemic control were included at the beginning of our study. Since the number of patients with SH was also small in our study, the relationship between thyroid dysfunction and diabetes complications could not be clearly established.

Due to the prevalence above, studies showing the relationship between metformin treatment and thyroid function have also gained considerable attention. Twenty-four patients with polycystic ovary syndrome, prediabetes, and untreated SH were treated with metformin (2.55 g/day) for 6 months, while bromocriptine treatment was added to half of the patients. It was reported that metformin decreased serum TSH levels but did not affect serum total and free thyroid hormone levels. It was also noted that the serum TSH-lowering effect of metformin was stronger in patients not treated with bromocriptine than those treated with bromocriptine, and this was weakly correlated with an improvement in insulin sensitivity. Ultimately, it has been suggested that metformin treatment may affect the regulation of thyrotropic function in hypothyroid patients by enhancing the action of thyroid hormone in the pituitary [45].

In order to explain how the metformin-induced reduction in serum TSH occurs, a number of hypotheses have been proposed, but the precise mechanism remains elusive. Metformin may alter the frequency and number of thyroid hormone receptors. Metformin has an inhibitory effect on hypothalamic AMPK activity in the liver, which can counteract T3 effects at the hypothalamic level by activating AMPK and suppressing hepatic gluconeogenesis [46]. The potential interaction of metformin and levothyroxine in the gastrointestinal tract seems unlikely because the serum TSH-lowering effect of metformin has also been observed in patients not treated with levothyroxine [47].

Different studies in patients with hypothyroidism and/or levothyroxine treatment have also shown a decrease in serum TSH levels under metformin treatment [48, 49]. In their retrospective study, Cappelli *et al*. also reported that metformin had a lowering effect on serum TSH in hypothyroid and euthyroid patients [50].

Rajput *et al*. showed that in patients with SH but without existing diabetes, metformin reduced serum TSH levels only in Anti-TPO positive subjects [51]. The results of the study by Dimic *et al*., which investigated the effect of metformin on serum TSH levels in euthyroid patients with a history of hypothyroidism and euthyroid patients with newly diagnosed T2DM, showed that 6 months of metformin treatment led to a statistically significant decrease in serum TSH level in the group with a history of hypothyroidism only. This decrease was greater than that in the euthyroid group. In the euthyroid group, meanwhile, a significant decrease in serum TSH levels was observed only in those who were anti-TPO positive. The results of this study suggest that metformin treatment has the effect of reducing serum TSH levels, but only in patients with chronic autoimmune thyroiditis and preexisting thyroid dysfunction [52]. In our prospective study, baseline serum TSH and anti-TPO levels of diabetic patients decreased after 6 months of follow-up with metformin treatment, and these decreases were found to be statistically significant. Thus, our study supports the results mentioned above. However, we could not measure thyroid autoantibodies in all our patients. Furthermore, since the number of patients who were anti-TPO positive and had high serum TSH levels was small, a comparison with other patients with normal levels could not be made. Jia *et al*. conducted a meta-analysis of these seven studies and divided them into two subgroups: SH patients and Hashimoto's thyroiditis patients. In terms of autoimmune antibodies, they found that metformin significantly reduced the level of anti-TPO, which was more pronounced in Hashimoto's thyroiditis group than in the SH group [53]. The levels of anti-TPO and anti-Tg are commonly used as indicators of Hashimoto's thyroiditis disease activity, of which anti-TPO is the most important indicator. Contrary to studies showing that metformin reduces

pathogenic antibodies in SLE disease [54]. Diaz *et al*. have shown that metformin can increase the protective antibody response to influenza vaccination in naïve T2DM patients [55]. Metformin activates AMPK phosphorylation, inhibits mTOR activity, and thus inhibits B cell activity, which is also associated with the inhibition of TNF-α, miR155 and miR-16 expression by metformin [56]. Our results also showed that anti-TPO levels detected in a small number of patients could be significantly reduced by metformin treatment.

Yet, some studies do not support the positive effects of metformin treatment on thyroid function in diabetic patients. According to the results presented by Diezz JJ, no association was found between serum TSH levels and metformin treatment in euthyroid T2DM patients [57]. Again, both Fournier *et al*. and Haroon *et al*. reported that the effect of metformin on serum TSH levels was shown only in a group of patients with hypothyroidism who received treatment. At the same time, it did not affect serum TSH levels in euthyroid patients [58, 59].

There are also studies investigating the effect of the duration of metformin use on thyroid function and arguing conflicting views. In a study, it was shown that the use of metformin for many years [>5 years) did not cause a significant decrease in serum TSH values in euthyroid newly diagnosed T2DM patients compared to 6 months of treatment [52]. Fournier *et al*. actually point out that the serum TSH-lowering effect of metformin is highest during the first 90 to 180 days of treatment [58]. Cappelli *et al*. also showed a more remarkable decrease in serum TSH levels in euthyroid subjects after 12 months of metformin treatment [50]. The duration of our study seems to be sufficient to demonstrate the effect of metformin treatment in lowering serum TSH levels.

In some of the studies investigating the effects of metformin treatment on thyroid volume and the presence of nodules, a decrease in thyroid nodule size after metformin treatment was shown in 66 female patients with IR [60]. This finding has also been supported in other studies [61, 62]. In our study, the mean baseline insulin level was 94.7 ± 62 pmol/L, and 79.4% had a baseline HOMA score of ≥ 2.5. Furthermore, all of our patients were T2DM patients. Despite this, it was observed that the presence of IR or diabetes in our patients did not significantly affect the thyroid volume at the beginning of the study (p = 0.894). However, with metformin treatment, the mean thyroid volume of our patients showed a statistically significant decrease at 6 months (p = 0.000). The study by Itterman *et al*. reported that no association was found between T2DM and increased thyroid volume or goiter. However, T2DM not treated with metformin was associated with a higher risk of goiter in the overall population (incidence rate ratio (IRR=1.70). Individuals who switched from metformin to other antidiabetic agents during follow-up had a higher risk of goiter compared to individuals without T2DM (IRR=2.71). Ultimately, metformin has an inhibitory effect on goiter. The anti-goitrogen effects of metformin also contribute to the overall benefits of metformin therapy in T2DM [13].

Although the relationship between obesity and metabolic outcomes related to thyroid function has been reported, the underlying pathogenesis is not fully understood [63-65]. Overall, in our study, a significant decrease in serum TSH levels was observed in non-obese patients who received metformin treatment for 6 months, while a significant decrease in thyroid volume was observed in obese individuals. Serum Anti-TPO levels showed a significant decrease both in those with and without obesity. Furthermore, in our study, baseline thyroid volume was slightly higher in obese patients compared to non-obese patients, and considering the presence of IR and related baseline thyroid volume in these patients compared to 6th-month levels, it can be suggested that metformin treatment could also diminish thyroid volume by reducing IR and obesity. Bodyweight and serum ALT levels also showed a statistically significant decrease in both obese and non-obese patients. The decrease in serum ALT levels was thought to be due to a reduction in hepatosteatosis, which was probably present at baseline with weight loss or metformin treatment.

In a study by Isidro *et al*., which aimed to prospectively evaluate whether the administration of metformin to obese diabetic patients with primary hypothyroidism at stable thyroxine replacement doses altered serum TSH levels, the mean serum TSH level decreased significantly 3 months after metformin treatment compared to baseline values. At 3 months after discontinuation of metformin treatment, TSH levels were not different from baseline values but elevated compared to the measurement 3 months after metformin treatment. Mean fT4 increased with metformin intake and decreased with discontinuation [66]. In a study aiming to evaluate thyroid parameters following metformin treatment in obese insulin-resistant patients with euthyroid, diffuse or nodular goiter, after 6 months, metformin added to patients treated with levothyroxine for diffuse/ nodular goiter showed a significant decrease in serum TSH and metabolic parameters compared to those treated with levothyroxine alone. Still, no significant difference was found in thyroid morphology after 6 months [67].

In a retrospective study, no difference was found between TSH concentrations in euthyroid patients with type 2 DM in treatments other than metformin (diet, oral antidiabetics, insulin, *etc*.). When comparing those who were started on metformin with those who were started on other antidiabetic drugs, it was shown that serum TSH levels remained significantly higher in those treated with other antidiabetic drugs such as sulfonylurea, meglitinide or thiazolidinedione. These antidiabetics did not have a significant effect on the decrease in TSH. However, it has been argued that metformin use is not associated with serum TSH levels after evaluating various potential confounding factors and that the presence of goiter may explain the difference in serum TSH levels between patients taking and not taking metformin, or conversely, that metformin may be an independent protective factor for goiter in T2 DM [57].

The main limitation of our study is that patients who could not be reached at the end of the 6th-month follow-up and whose control examinations could not be seen caused incomplete data. Besides, the positive or negative effects of these medications on the efficacy of metformin in patients with additional drug use could not be evaluated due to the insufficient number of patients. Since the incidence of thy-

roid dysfunction was low in our patients, it was not possible to fully evaluate the efficacy of metformin treatment on normal or impaired thyroid function. There is a need for further prospective, controlled, randomized trials to gain additional insight into this issue.

CONCLUSION

In conclusion, our study revealed that metformin treatment significantly decreased body weight, BMI, thyroid volume, and serum TSH, ALT, and Anti-TPO levels in patients with newly diagnosed T2DM. Moreover, serum TSH levels showed a significant decrease in non-obese subjects, while thyroid volume showed a significant decrease in obese subjects. The obtained data strongly support that changes in thyroid hormones may be affected by adiposity and its metabolic consequences, such as IR. Besides, metformin treatment seems to affect thyroid function in diabetic patients by maintaining serum TSH levels to subnormal levels. As the rate of newly diagnosed patients with T2DM is high and the potential impact of undiagnosed thyroid dysfunction is great, it is imperative to make routine thyroid function tests for patients with T2DM. The favorable effects of metformin treatment on thyroid function and thyroid volume in T2DM patients should be supported by more comprehensive studies.

AUTHORS' CONTRIBUTIONS

ME performed and designed the research; ME and GP followed the patients and researched data; İBG performed the ultrasonography; GP collected the data; ME and GP wrote the paper. Final approval: all authors.

LIST OF ABBREVIATIONS

AMPK	=	AMP-activated Protein Kinase
BMI	=	Body Mass Index
IFG	=	Impaired Fasting Glucose
IGF-1	=	Insulin-like growth factor-1
IR	=	Insulin Resistance
MAPK	=	Mitogen-activated Protein Kinase
PCOS	=	Polycystic Ovary Syndrome
SH	=	Subclinical Hypothyroidism
TSH	=	Thyroid Stimulating Hormone
TSHR	=	Thyroid Stimulating Hormone Receptor

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Çukurova University Ethics Committee (date: April 13, 2018, meeting number: 76, decision no: 101).

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committees and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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