

Original Article

EVALUATION OF CLINICAL PARAMETERS AS EARLY DIAGNOSTIC MEASURES FOR POSSIBLE COMPLICATIONS RESULTING FROM HYPOTHYROIDISM DERIVED FROM DIABETES

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ABSTRACT

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Type 2 diabetes mellitus (T2DM) has an interconnected physiological association with thyroid dysfunction (TD), leading to a higher prevalence of TD in T2DM patients than non-diabetic individuals. Abnormal thyroid hormones are associated with the onset of diabetes complications. This study aimed to investigate the implication of thyroid hormones, hyperglycemia, liver enzymes, renal function test, and lipid profile as predictive and diagnostic biomarkers of diabetes complications in this population. This case-control study was conducted in Zakho, Iraq. It recruited 108 participants (54 cases and 54 controls). Data were obtained through a questionnaire, and blood specimens were collected from study participants for clinical tests. Patients with diabetes induced hypothyroidism were older and mostly females 49 (90.74%), compared with controls. Glycated hemoglobin (HbA1c), glucose, body mass index (BMI), thyroid hormones except free thyroxine (FT4), cholesterol, urea, and aspartate aminotransferase (ALT) levels, and some hematological parameters showed significant differences ($p < 0.05$). HbA1c showed significant positive correlation with duration of DM, glucose, age, BMI, thyroid-stimulating hormone (TSH) and urea and inversely correlated with free triiodothyronine (FT3) and FT3/FT4 ratio. Also, urea and ALT are indicated as potential predictors of the onset of diabetes complications in diabetic hypothyroid patients. The results concluded that hypothyroidism in T2DM can cause uncontrolled hyperglycemia, unstable thyroid hormone levels, and the progression and onset of diabetes complications. It further supported the strong correlation between hyperglycemia and thyroid hormones. Further studies are required to ascertain the precise mechanism by which high blood TSH and uncontrolled hyperglycemia cause renal impairment, liver damage, and cardiovascular disease, as well as the prevalence of microvascular complications among diabetic individuals with hypothyroidism.

Keywords: Diabetes mellitus, Diabetes complications, Hypothyroidism, Lipid profile, Liver function, and Renal function.

1. INTRODUCTION

Diabetes mellitus (DM) is a long-term, metabolic and endocrine disorder (Demir *et al.*, 2021). Increased blood glucose level is the main characteristic of this chronic condition and leads to impairments in protein, carbohydrate, and lipid metabolism (Vemula *et al.*, 2023a). DM is related to impaired insulin production, poor insulin action, or both (Punthakee *et al.*, 2018). Since 1980, the prevalence of this chronic condition has almost doubled worldwide (Li *et al.*, 2022), increasing in the adult population from 4.7% to 8.5% (Biondi *et al.*, 2018). In the general population, thyroid dysfunction (TD) is a system-affecting, second-most-frequent endocrine disease after diabetes (Ogbonna & Ezeani, 2019). The most prevalent types of TD are hypothyroidism and hyperthyroidism (Sakya *et al.*, 2023). There exists a profound and enduring correlation between DM and TD, this correlation dates back to 1979 (Wang, 2013; Vemula *et al.*, 2023b). Therefore, it is typical for a person to suffer from both diabetes and thyroid conditions (Ogbonna & Ezeani, 2019). People with diabetes

typically develop TD, which affects their metabolic status (Elumalai *et al.*, 2024), especially those with poor glycemic control are more likely to develop TD (Kadiyala *et al.*, 2010). About 10% to 15% of people with diabetes have TD, while non-diabetics have a frequency of around 6% (Hadgu *et al.*, 2024). A case-control study involving diabetic Kurdish females and age- and sex-matched controls reported that primary hypothyroidism was found to be 40% more common in T2DM patients and 10% in controls (Muhammed & Albustani, 2018). Patients with diabetes may experience weight gain, dyslipidemia, and a higher degree of insulin resistance as a result of hypothyroidism, which can negatively affect their metabolic profile (Elumalai *et al.*, 2024). Because thyroid hormones (TH) are insulin antagonists and indirectly affect insulin activity, they are crucial for insulin sensitivity. Hence, dysfunction in the metabolism of each hormone can lead to functional abnormalities in the other. On the other hand, insulin is an anabolic hormone that is important for increasing the value of FT4 and suppressing the level of FT3 by preventing the liver from converting thyroxine to triiodothyronine (Taher, 2024). Also, THs affect

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lipid metabolism, especially by increasing the rate of cholesterol synthesis through the enzyme HMG-CoA reductase (enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase) and, consequently, upregulate low-density lipoprotein (LDL) receptor expression in the liver to encourage cholesterol elimination (Biondi *et al.*, 2018).

Since each of these endocrinopathies and their many interdependent relationships raise the risk of microvascular complications, it is crucial to test for TD in individuals with T2DM (Kalra *et al.*, 2019). Therefore, this study aims to investigate the association between various clinical parameters in post-diabetes hypothyroidism (diabetic hypothyroid patients) and evaluate the use of these parameters as prognostic and diagnostic markers of the complications arising from the coexistence of these conditions. The novelty of this study lies in the assessment of the key clinical parameters in patients with diabetes-induced hypothyroidism, which will contribute to improving the understanding of diabetic hypothyroidism, its management, pathophysiology, and early diagnosis of its complications.

2. MATERIALS AND METHODS

Study design

The participants in the current investigation were separated into two groups: 54 cases and 54 controls. Both groups were age- and sex-matched. This case-control study was designed to investigate the serum levels of thyroid function hormones, lipid profile biomarkers, renal function biomarkers, liver function biomarkers, fasting glucose, Glycated hemoglobin (HbA1c), and complete blood count in cases and controls. The sample collection was conducted over five months, from August to December (2024), at different hospitals and medical centers in Zakho city.

Inclusion and exclusion criteria

The cases were on medication patients for both diabetes and thyroid dysfunction (hypothyroidism), and the controls were biochemically confirmed to be healthy in every test performed. Patients with cardiovascular diseases, chronic liver diseases, and chronic kidney diseases were excluded, as well as pre-diabetics, pregnant, and breast-feeding women.

Data collection and clinical tests

Data were obtained through a comprehensive questionnaire. For collecting the peripheral blood specimens, the standard phlebotomy method was used. 8 ml of fasting venous blood was collected from each participant, 2 ml in EDTA tubes used for HbA1c measurement and complete blood count, and 6 ml in gel tubes used for the separation of serum. After being separated by centrifugation at 10000 rpm for five minutes, sera were stored at -70°C for biochemical tests.

Cobas 6000 c501 autoanalyzer system (Roche Diagnostics, HITACHI) was used to measure FBG (fasting blood glucose), lipid profile; cholesterol, triglycerides, and high-density lipoprotein (HDL), renal function biomarkers (urea, creatinine, uric acid), and liver function markers ALT (alanine transaminase), AST (aspartate transaminase), and GGT (gamma-glutamyl transferase). Whereas, the Cobas 6000 c601 autoanalyzer system (Roche Diagnostics, HITACHI) was utilized to measure each participant's TSH (thyroid-stimulating hormone), FT3 (free triiodothyronine), and FT4 (free thyroxine) levels. Hematology autoanalyzer (Medonic Coulter Counter, Sweden) for Complete blood count (CBC) and Cobas 311 autoanalyzer system (Roche Diagnostics, HITACHI) for HbA1c (Hemoglobin A1c) measurement were used. Instead of taking direct measurements, the Friedewald equation was used to determine the levels of low-density lipoprotein (LDL) and very-LDL.

Data Processing and Statistical Analysis

Kolmogorov-Smirnov test and QQ plots method were used to check the normality of numerical data. All selected parameters (vari-

ables) were presented as mean $\pm$ standard deviation (SD), or numbers (%). To assess the association between categorical data, the Chi-Square test was used. By using the Mann-Whitney U test, the clinical parameters were compared between cases and controls. Additionally, to analyze the relationship between clinical indicators, Spearman correlation analysis was used. Moreover, the diagnostic performance of each biomarker in predicting complications associated with diabetic hypothyroidism was assessed using the Receiver Operating Characteristic (ROC) curve. For evaluation of each marker's capacity for discrimination, the area under the curve (AUC) was computed. The Youden Index was used to calculate the ideal cut-off values. SPSS software (Statistical Package for the Social Sciences) was used to analyze data. A p value < 0.05 was considered statistically significant, and a p value < 0.0001 was used for highly significant differences.

3. RESULTS

Demographic characteristics

Table 1 presents number and percentages of gender, age groups, and BMI status for both diabetic patients with hypothyroidism and non-diabetic individuals. When demographic characteristics of the patients and controls were compared, Chi-square results indicated that there was a significant difference between sex ($p = 0.021$). Most of the patients were women 49 (90.74%), which indicates that the coexistence of DM with hypothyroidism was more prevalent in women. Also, a significant difference in age group distribution between cases and controls was found ($p = 0.002$). Additionally, notable differences were found between BMI status in both groups ($p = 0.006$). Most of the patients were either overweight 14 (25.93%) or obese 36 (66.67%).

Table 1: Comparison of demographic characteristics between diabetic hypothyroid patients and controls.

	Controls No.54	Cases No.54	p-value	Chi-square
Gender				
Female	39 (72.2%)	49 (90.74%)	0.021*	7.684
Male	15 (27.8%)	5 (9.26%)		
Age groups				
<40 (Year)	7 (13%)	4 (7.4%)	0.002*	12.250
40-60 (Year)	41 (75.9%)	31 (57.4%)		
>60 (Year)	6 (11.1%)	19 (35.2%)		
BMI status				
Normal	14 (25.93%)	4 (7.4%)	0.006*	10.237
Overweight	25 (46.30%)	14 (25.93%)		
Obese	15 (27.78%)	36 (66.67%)		

Chi-square test. *= Parameters with 2-tailed sig < 0.05 . NO (Number of individuals in the group).

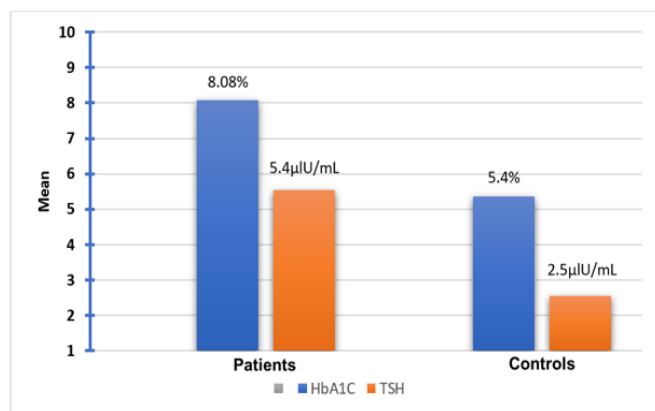
Baseline and clinical parameters of the examined population

The clinical parameters of both study groups are summarized in Table 2. The two groups differed significantly in terms of age and BMI distribution ($p < 0.0001$). The biochemical parameters comparing cases with DM and TD and controls revealed numerous significant differences. In cases, HbA1c, TSH, and were significantly higher in patients ($p < 0.0001$ for both) (Figure 1). Also, urea levels were significantly higher in comparison with the control group ($p = 0.001$). However, cholesterol, FT3, FT3/FT4 ratio, and ALT serum levels were substantially lower in the patients ($p = 0.031$, $p < 0.0001$, $p = 0.0004$, and $p = 0.012$, respectively). But there were no significant differences for other clinical parameters between the two groups.

Table 2: Clinical and biochemical parameters of study groups.

Parameters	Controls	Cases	p-value
HbA1c (%)	5.4 ± 0.3644	8.08 ± 1.7	<0.0001*
Age (Years)	46.5 ± 6.8	55 ± 11.05	<0.0001*
FBG (mg/dL)	98.5 ± 17.1	159 ± 65	<0.0001*
BMI (kg/m ²)	28.9 ± 4.6	32.9 ± 5.8	<0.0001*
<i>Thyroid function profile</i>			
TSH (uIU/mL)	2.5 ± 2.5	5.4 ± 0.7	<0.0001*
FT3 (pmol/L)	6.3 ± 0.6	5.7 ± 38.5	<0.0001*
FT4 (pmol/L)	15.5 ± 2.1	15.7 ± 2.7	0.378
FT3/FT4 ratio	0.41 ± 0.05	0.36 ± 0.08	0.0004
<i>Lipid profile</i>			
Cholesterol (mg/dL)	193.5 ± 37.4	177.7 ± 38.8	0.031*
Triglycerides (mg/dL)	189.9 ± 109.4	187.7 ± 93.4	0.654
HDL (mg/dL)	40.9 ± 9.5	48.2540 ± 5.03	0.078
LDL (mg/dL)	117.01 ± 41.15	120.95 ± 18.5	0.134
VLDL (mg/dL)	37.3 ± 22.3	38.44 ± 18.6	0.656
<i>Renal function tests</i>			
Urea (mg/dL)	26.2 ± 6.2	32.3 ± 11.4	0.001*
Creatinine (mg/dL)	0.7 ± 0.2	0.8 ± 0.3	0.808
Uric acid (mg/dL)	4.5 ± 1.2	4.4 ± 1.2	0.704
<i>Liver function tests</i>			
ALT (U/L)	13.9 ± 6.5	11.7 ± 7.5	0.012*
AST (U/L)	19.9 ± 5.6	18.6 ± 6.2	0.125
GGT (U/L)	26.6 ± 20	33.3 ± 24.3	0.182

Mann-Whitney U Test, results are represented as means ± SD. *= Parameters with 2-tailed sig < 0.05.

**Figure 1:** Bar chart, representing HbA1c and TSH differences between cases and controls.

Additionally, from hematological parameters represented in Table 3, LYM, MID, HCT, RDW, and PDW levels were significantly higher ($p < 0.05$) in cases and lower levels were recorded in the control group. In contrast, significant lower levels of GRA ($p = 0.015$) and MCH ($p = 0.018$) were observed in patients, and higher levels in controls.

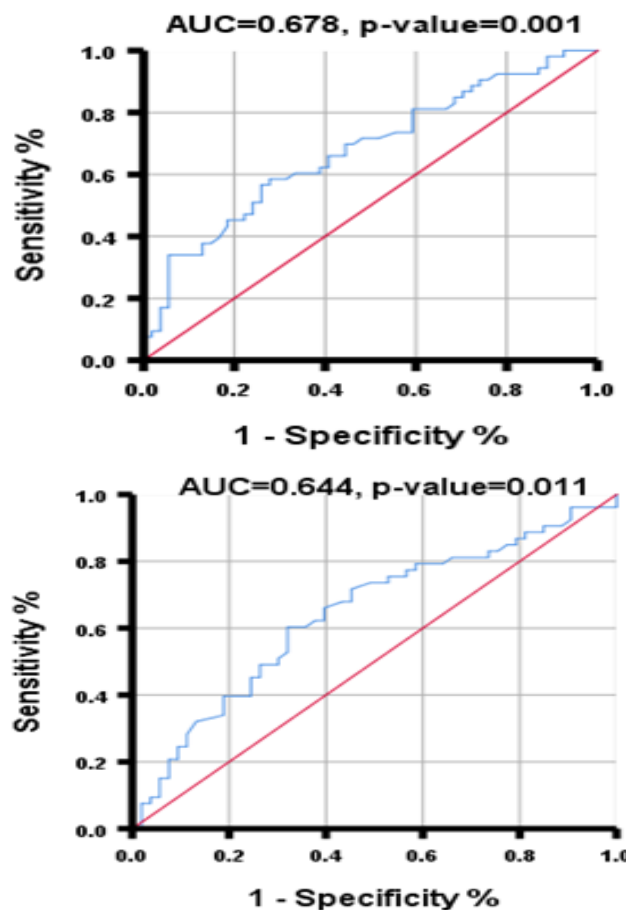
Table 3: Hematological parameters of diabetic hypothyroid patients and controls are compared.

Parameters	Controls	Cases	p-value
WBC ($\times 10^9/L$)	6.9 ± 2.5	7.3 ± 2.2	0.175
LYM ($\times 10^9/L$)	2.9 ± 1.4	4.2 ± 1.6	<0.0001*
MID ($\times 10^9/L$)	0.4 ± 0.3	2.1 ± 9.3	<0.0001*
GRA ($\times 10^9/L$)	3.5 ± 2	2.5 ± 2.1	0.015*
RBC ($\times 10^9/L$)	4.8 ± 0.6	5 ± 1.4	0.486
HGB (g/dL)	13.4 ± 1.3	13.3 ± 2.4	0.270
HCT (%)	39.2 ± 5	42.3 ± 6.2	0.025*
MCV (fl)	834 ± 9.3	86.9 ± 16.7	0.071
MCH (pg)	28.3 ± 2.8	28.2 ± 10	0.018*
RDW (fl)	55 ± 10.1	70.563 ± 30.5	0.010*
PLT ($10^9/L$)	204.7 ± 74.5	209.1 ± 98	0.997
MPV (fl)	10.2 ± 1.2	10.8 ± 2.6	0.065
PCT (%)	0.2 ± 0.09	0.6 ± 6.8	0.439
PDW (fl)	13.5 ± 4.3	15.7 ± 6.8	0.031*

Mann-Whitney U Test, results are represented as means ± SD. *= Parameters with 2-tailed sig < 0.05.

3.1 The predictive value of clinical parameters

The current study used ROC curve and area under the curve (AUC) to evaluate the predictive value of urea and ALT for diabetic hypothyroid dysfunction complications, as shown in Figure 2 (A). With a substantial cut-off value ≥ 38.8 mg/dl, AUC= 0.678, a sensitivity of 63.9%, and a specificity of 60% ($p = 0.001$; Confidence interval (CI) 95%: 0.577 to 0.779), urea demonstrated noteworthy predictive performance. In Figure 2 (B), with an optimum cut-off value of ≤ 9.95 , AUC= 0.644, a sensitivity of 71.2%, and a specificity of 58% ($p = 0.011$; CI 95%: 0.538 to 0.751), ALT also demonstrated promising results.

**Figure 2:** A prognostic analysis of the serum levels of (A) urea and (B) ALT in diabetic hypothyroid patients is presented.

Association between HbA1c and clinical characteristics

Results from Spearman correlation analysis revealed a series of significant correlations between HbA1c and clinical parameters, as presented in Table 4. HbA1c showed a highly significant positive correlation with duration of DM, FBG, age ($p < 0.0001$ for all) and BMI ($p = 0.002$). These findings suggest that as the duration of DM increases and a person gets older, HbA1c level significantly increases, and elevated HbA1c is associated with elevated FBG level. For thyroid hormones, HbA1c was significantly positively correlated with TSH ($p = 0.009$) and showed an inverse relationship with FT3 and FT3/FT4 ratio ($p < 0.0001$ and $p = 0.004$, respectively). The correlation of HbA1c with renal, liver, and lipid profile biomarkers revealed no significant associations between HbA1c and all given parameters, except for urea; HbA1c showed a significant positive association with serum urea level ($p = 0.002$).

Table 4: Correlation between clinical parameters, with corresponding p-value and correlation coefficients.

HbA1c	r	p-value
Duration of DM	0.450**	<0.0001*
FBG	0.577**	<0.0001*
Age	0.393**	<0.0001*
BMI	0.300**	0.002*
<i>Thyroid function profile</i>		
TSH	0.259**	0.009*
FT3	-0.392**	<0.0001*
FT4	0.051	0.613
FT3/FT4 ratio	-0.285**	0.004*
<i>Liver function tests</i>		
ALT	-0.136	0.179
AST	-0.119	0.239
GGT	0.123	0.226
<i>Renal function tests</i>		
Urea	0.307**	0.002*
Creatinine	-0.069	0.498
Uric acid	-0.0074	0.462
<i>Lipid profile</i>		
Cholesterol	-0.117	0.252
Triglycerides	0.030	0.769
HDL	0.211	0.137
LDL	0.139	0.171
VLDL	0.03	0.769

Spearman Correlation Analysis, * = parameter with 2-tailed sig. (p-value) < 0.05, r = Spearman Correlation Coefficient.

4. DISCUSSION

According to statistical analysis, diabetic hypothyroidism was more common in women and is substantially linked to obesity and advanced age, this was attributed to the role of age as a risk factor for both diabetes and hypothyroidism. These findings are parallel with previous studies that stated hypothyroidism has been demonstrated to be more common in patients with type 1 diabetes mellitus (T1DM) and T2DM, particularly in those with risk factors such as female sex, obesity, hyperlipidemia, and anemia (Faruque *et al.*, 2022; Alqahtani & Alshehri, 2022; Vemula *et al.*, 2023a). Also, there were highly significant differences in HbA1c and fasting glucose levels between diabetic hypothyroid patients and controls. This finding aligns with most of the previous works, but contrasts with the results obtained from an observational study by Gurunath *et al.* (2019). Moreover, our analysis of hematological parameters indicated that LYM and MID numbers were significantly higher in patients, and, HCT, RDW, and PDW of patients were elevated. These increases can suggest mixed anemia or early changes in RBC production. These pathophysiological alterations are the result of increased high generation of internal pro-inflammatory markers and systemic inflammation, in chronic disease, diminished erythropoietin production and release, as well as altered iron metabolism, impairing red blood cell creation (Alqahtani & Alshehri, 2022).

It has been established that THs play a major part in both pancreatic and carbohydrate metabolism. Likewise, it has been demonstrated that diabetes affects TD to varying degrees (Santosh *et al.*, 2023). Our results also showed a substantial difference in TH levels between patients and their nondiabetic control counterparts. Since all of the cases had post-diabetic hypothyroidism, it is widely believed that uncontrolled hyperglycemia is the cause of these hormonal changes. According to Ogbonna and Ezeani (2019), the risk of the development of TD is 4.3 times higher in T2DM patients with (HbA1c < 7%), and there is a significant association between THs and diabetes indicators, which strongly aligns with our finding, HbA1c was significantly positively associated with serum TSH levels. There is conflicting evidence linking hyperlipidaemia with overt hypothyroidism and even subclinical hypothyroidism to alterations in plasma lipids (Biondi *et al.*, 2018). Our case-control comparison results revealed a substantial difference in the serum cholesterol levels between both groups, with

the lower levels of cholesterol observed in patients than in controls, which could be attributed to the combined effects of levothyroxine sodium and diabetes medications.

A review study stated that thyroid gland disorders or, less frequently, TH use may be the cause of liver damage linked to hypothyroidism (Yorke, 2024). Our study found that patients had significantly lower serum ALT levels compared to controls. In older diabetic patients with below-normal ALT levels, this finding may indicate underlying metabolic unhealthiness. Additionally, low ALT levels in elderly adults are associated with frailty (Chung *et al.*, 2020).

Furthermore, some investigations have discovered a connection between TH levels and a range of chronic complications of diabetes, including diabetic retinopathy and diabetic nephropathy (He *et al.*, 2023). THs have an impact on renal physiology and development, they can also raise renal blood flow, which lowers GFR, especially in diabetic patients (Nsr-Allah *et al.*, 2018). The current study results reported that the serum levels of urea were significantly increased in patients, and a significant association was found between HbA1c and serum urea level. Our results are in agreement with the findings of Nsr-Allah *et al.* (2018), who conducted a study on T2DM patients and found that subclinical hypothyroidism and diabetic nephropathy (DN) are significantly associated. So, to prevent DN or further decline in kidney function, it is advised that all patients with type 2 diabetes undergo routine thyroid function tests.

Additionally, the key innovative findings of the current study emphasize the potential of urea and ALT as tools in assessing the risk of microvascular problems in diabetic hypothyroid individuals, as well as predicting markers for the emergence of diabetes-related complications in patients with coexisting T2DM and hypothyroidism. These findings highlight the need for further study to examine the diagnostic importance of urea and ALT in diabetic hypothyroid patients, as direct comparisons with existing data are difficult due to the limited comparative research accessible in the literature. A longitudinal population-based cohort study indicated that low ALT cut-off values were associated with increased risk of liver-related mortality and decompensated liver events (Park *et al.*, 2019). The study has a few limitations. Firstly, the limited sample size ($n = 108$) may affect the generalizability of the results. Additionally, the cross-sectional approach restricts the capacity to determine causality between hypothyroidism and diabetes problems. Furthermore, the absence of molecular investigations limits a comprehensive understanding of the pathways linking thyroid hormones to diabetic complications, which could provide insights into how these conditions interact and influence each other.

5. CONCLUSION

This analysis reveals that diabetes-induced hypothyroidism is more common in females than in males, particularly among older age groups and individuals with a higher BMI. Also, this study emphasizes the critical relationship between hypothyroidism and diabetes mellitus, indicating that persons with hypothyroidism are at an elevated risk of uncontrolled hyperglycemia and its related consequences. Chronic hyperglycemia, coupled with thyroid dysfunction, may lead to microvascular complications in diabetic patients with hypothyroidism. A thorough understanding of the interplay between Type 2 Diabetes Mellitus (T2DM) and thyroid dysfunction is vital for enhancing clinical outcomes and reducing cardiovascular risks linked to these conditions. Future studies should focus on larger, longitudinal research that incorporates molecular analyses to better understand the connections between thyroid dysfunction and diabetes complications. By clarifying the mechanisms involved, researchers can evaluate how the regulation of thyroid hormones affects diabetes outcomes and discover potential pharmaceutical targets for preventing or managing complications in diabetic individuals with hypothyroidism.

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ETHICAL APPROVAL AND SUBJECT RECRUITMENT

The Research Ethics Committee, College of Medicine, UOZ, provided the ethical approval letter to the current study (Trial registration: OCT2024/UOZ18). All participants received thorough information about the goals of the study and any possible risks before their involvement. Each participant then signed a consent form to give their informed consent.

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Author Contribution:

Both authors made contributions to the design and idea of the investigation. R.N. contributed to the data collection and analysis, wrote the article, and analyzed the findings. The concept was created by C.M., who also oversaw the research and gave her approval to the manuscript's final draft.

Consent for publication:

Not applicable.

Competing interests:

The authors declare no competing interests.

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