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The Predictive Value of Melatonin Levels for the Development of Diabetic Nephropathy in Men with Type 2 Diabetes Mellitus

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Abstract

Background: Type 2 diabetes mellitus (T2DM) poses a significant public health challenge due to its high prevalence. Diabetic nephropathy (DN) is one of the most severe complications associated with T2DM. Early prediction of DN in patients with T2DM can significantly aid in managing this disease. This study takes an approach by investigating the potential role of melatonin and thyroid hormone levels as predictive biomarkers for the progression of diabetic nephropathy in individuals diagnosed with type 2 diabetes mellitus.

Methods: Our cross-sectional study involved 120 male participants, divided into two groups: 60 patients with T2DM and 60 with DN. The Cobas technique was used to measure serum thyroid hormone levels and quantified melatonin levels using an enzyme-linked immunosorbent assay (ELISA). A receiver utilizing characteristic (ROC) curve analysis to evaluate the predictive value of serum melatonin for DN was performed.

Results: No notable disparities in thyroid function tests were observed between diabetic patients with and without DN. However, the average serum melatonin quantity in patients with DN. (177.25 ± 60.48 pg/mL) was drastically lower in those with T2DM without DN (199.9 ± 55.16 pg/mL). The sensitivity and specificity of melatonin in predicting DN were 78% and 76%, respectively, with an optimal cut-off value of 178 pg/mL.

Conclusions: Serum melatonin levels exhibited a notable reduction. among individuals who were diabetic with DN, suggesting its potential utility as an additional predictive marker for developing DN in patients with T2DM.

Keywords: Diabetes Mellitus, Diabetic Nephropathy, Melatonin, Thyroid Activity Tests.

Introduction

Diabetes mellitus (DM) is a highly prevalent and potentially life-threatening metabolic disorder characterized by elevated blood glucose levels resulting from either impaired insulin secretion or insulin resistance (1). Among the various complications associated with diabetes, diabetic nephropathy (DN) emerges as a significant microvascular complication, contributing substantially to the morbidity and mortality of individuals with diabetes (2,3). DN arises from vascular abnormalities linked to diabetes and is a leading cause of end-stage renal disease (ESRD) and diabetes-related health complications worldwide (4). Diabetes alone accounts for 12% to 55% of ESRD cases (2).

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Microalbuminuria (MA), a urinary albumin excretion rate (UAE) ranging from 30 to 300 mg/day, is an early and widely utilized clinical marker for DN. Diabetes mellitus (DM) independently correlates with cardiovascular risk in diabetic patients (5), mainly reflecting the presence of widespread microvascular damage and underlying renal impairment.

Melatonin (N-acetyl-5-methoxytryptamine) is an endogenous neurohormone derived from tryptophan and synthesized in the pineal gland. Renowned for its potent antioxidant properties, melatonin effectively neutralizes various free radicals while enhancing antioxidant enzyme expression and suppressing inflammatory protein production across cell types (6). Structural features of melatonin, including its electron-richness, hydrophilicity, and lipophilicity, contribute to its potential impact on antioxidant capacity and modulation of insulin secretion through the MT1 receptor (7).

Thyroid hormones are crucial in metabolism, energy homeostasis, and glucose regulation (8, 9). Elevated levels of thyroid-stimulating hormone (TSH) and reduced levels of free triiodothyronine (FT3) have been associated with an increased risk of chronic kidney disease (CKD) (10,11). Furthermore, in patients with T2DM, low serum FT3 levels have been independently correlated with proteinuria (12).

This study aimed to evaluate the potential predictive roles of endogenous melatonin and thyroid hormone levels in the development of DN in patients with T2DM.

Materials and Methods

Subjects and samples

This cross-sectional study included 120 male patients with Type 2 Diabetes Mellitus (T2DM) aged 25-79 years. He research was conducted at AL-Karama Teaching Hospital, AL-Kindy Teaching Hospital, and AL-Yarmouk Teaching Hospital in Baghdad, Iraq, from August 2023 to December 2023. The participants were divided into two groups: 60 patients with T2DM and 60 patients with DN undergoing hemodialysis treatment three times a week for three hours per session.

A comprehensive medical history, including age, weight, height, body mass index (BMI), family history of diabetes, comorbidities, fasting blood sugar (FBS), and glycated hemoglobin (HbA1c), was collected from each participant.

Blood samples, approximately 5 ml, were collected in a plain tube, before administering the heparin dose for those undergoing hemodialysis, without using a tourniquet. The samples were centrifuged 1006 G for 10 minutes to obtain serum. The samples were frozen at -20 °C until utilized for clinical tests.

Biochemistry tests

Ready commercial kits (Elecsys/ Roche Diagnostics GmbH, Germany) were used to measure T3, T4, and TSH hormone serum levels. The Cobas e 411 analyzer is a completely automated analyzer that conducts immunoassay analysis by utilizing patented Electro Chemi Luminescence (ECL) technology. A ready-to-use commercial kit (Melsin/China) was used to measure serum melatonin levels via enzyme-linked immunosorbent assay (ELISA). The company's instructions were precisely followed.

Statistical analysis

The statistical analyses were performed using the SPSS 25.0 program (SPSS, Chicago). Continuous data were presented as mean and standard deviation, and the Student’s t-test was used for analysis. The predictive efficacy of melatonin in predicting diabetic nephropathy (DN) among patients with type 2 diabetes mellitus (T2DM) was assessed using the receiver operating characteristic (ROC) curve. Pearson's correlation test explored potential correlations between melatonin, thyroid function tests, and other variables. A p-value less than 0.05 was considered statistically significant.

Results

The average age of patients in the DN group was 54.35±10.83 years, which was not significantly different from the 51.6±5.41 years of the diabetic patients. For diabetic patients

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with and without DN, there was no significant difference in BMI similar to age; nevertheless, the BMIs of both groups were significantly higher than those of the controls ($p<0.001$). As a marker for diabetic control, HbA1c was higher in patients with diabetes (8.31±1.34%) than in patients with DN (6.62±1.81%), with highly significant differences. However, FBS did not differ significantly between the DN and diabetic groups (181.32±44.2 mg/dl and 181.68±39.41 mg/dl, respectively). The median duration of hemodialysis in patients with DN was 14.5 months (Table 1).

Table 1. Demographic and clinical characteristics of those who were being examined

Variable		Diabetic neuropathy (n=60)	Diabetes mellitus (n=60)	p-value
Age (year)	**Mean±SD**	54.35±10.83	51.6±5.42	0.084
	Range	25-71	44-60	
Weight (kg)	**Mean±SD**	81.41±8.84	78.85±11.86	0.383
	Range	44-105	62-100	
Height (cm)	**Mean±SD**	170.0±8.62	164.75±8.07	0.121
	Range	145-185	155-182	
BMI (Kg/m^2)	**Mean±SD**	27.26±5.82	28.95±3.08	0.218
	Range	16.16-46.81	24.72-36.73	
FBS (mg/dl)	**Mean±SD**	181.89±45.92	181.68±39.41	0.980
	Range	133-320	130-248	
HbA1c	**Mean±SD**	6.62±1.81	8.31±1.34	<0.001
	Range	4.3-11.5	6.3-11.3	
HD duration	**Median**	14.5	-------	-------
	Range	2.0-144		

Overall, the two groups had no significant differences in thyroid function tests, and almost all of these tests were within the normal range (Table 2).

The mean serum level of melatonin in patients with DN was 177.25±60.48 pg/ml, significantly lower than that of patients with DM (199.9±55.16 pg/ml), with a significant difference (Fig. 1). A receiver operating characteristic (ROC) curve was used to evaluate the predictive value of melatonin in predicting DN in patients with DM. The area under the curve (AUC) was 0.713, 95% CI = 0.61-0.817, $p<0.001$. The sensitivity and specificity of the test at the cut-off value of melatonin = 178 pg/ml were 78% and 76%, respectively (Fig. 2).

Pearson's correlation was used to explore the possible correlation of thyroid function tests and melatonin with other variables. Apart from the highly significant correlations between components of thyroid function tests, there were no significant correlations between different variables (Table 3).

The correlations and distributions of melatonin in the column, as well as the linear relationships between variables. The diagonal plots provide insights into each variable's distribution, indicating whether it follows a normal distribution or has any outliers (Fig. 3).

Melatonin vs. Kidney Function Markers: The scatterplots in the off-diagonal panels depict the relationships between melatonin and various biomarkers, such as FBS, T3, and T4.

These plots indicate that as melatonin levels increase, there are corresponding changes in these kidney function parameters. The patterns suggest an inverse relationship between melatonin and markers. The clustering observed in the scatterplots suggests distinct subgroups within the study population, potentially based on disease severity. These subgroups exhibit different melatonin-kidney function relationships, which could be

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important for understanding the role of melatonin in the pathogenesis and progression of diabetic nephropathy.

Overall, this pair plot analysis provides valuable insights into the potential association between melatonin levels and various markers in individuals with diabetes. The observed patterns warrant further investigation, such as examining the underlying mechanisms and the possible therapeutic implications of melatonin supplementation and exploring the clinical relevance of the identified subgroups.

Table 2. Renal function test of the study population.

Variable		Diabetic neuropathy (n=60)	Diabetes mellitus (n=60)	p-value
TSH (mU/ml)	Mean±SD	2.24±1.65	2.05±1.33	0.502
	Range	0.04-8.7	0.37-7.16	
T3 (µg/dl)	Mean±SD	1.43±0.39	1.41±0.36	0.791
	Range	0.71-2.3	0.76-2.81	
T4 (µg/dl)	Mean±SD	9.67±2.08	10.0±2.35	0.436
	Range	5.93-14.8	5.8-19.78	

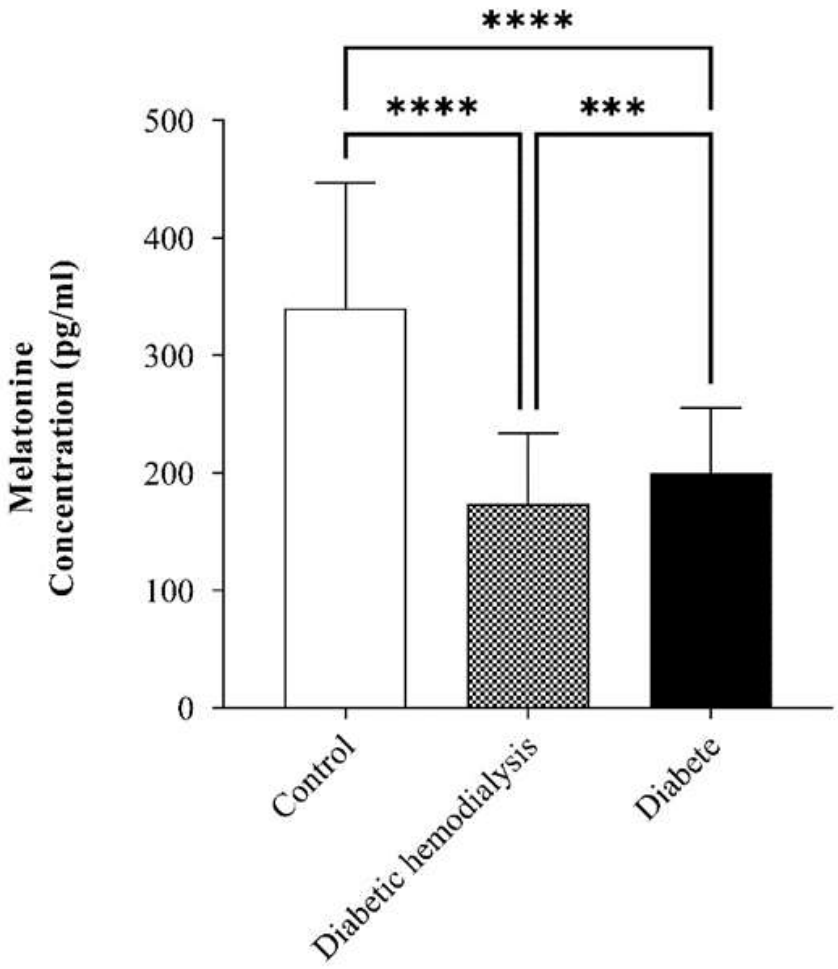

Fig. 1. Mean serum level of Melatonin in patients with DN and DM.

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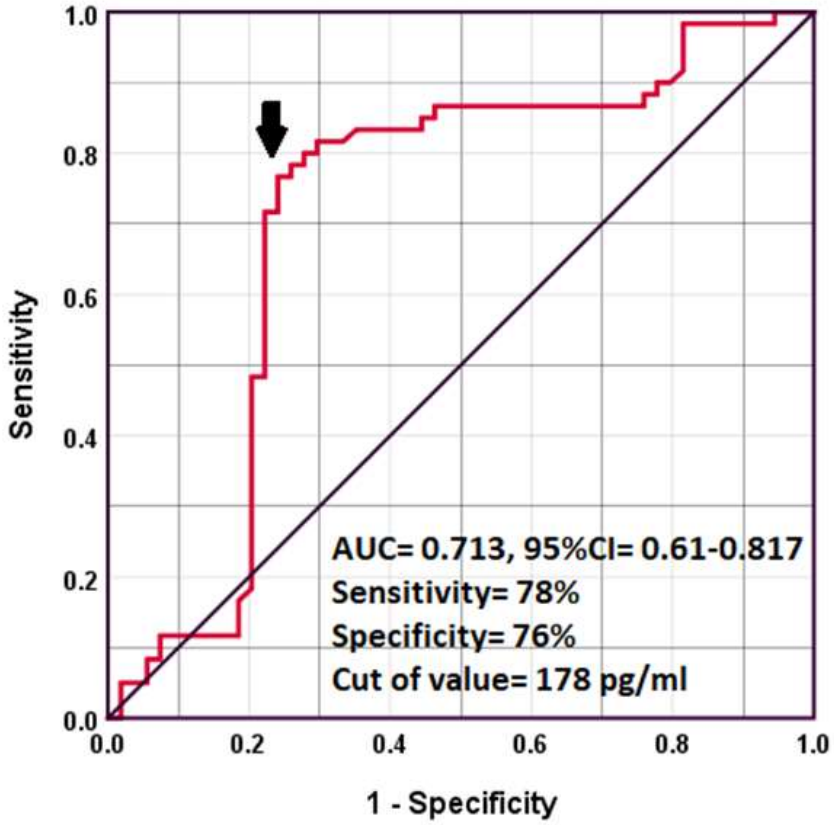

Fig. 2. Receiver operating characteristic curve for melatonin in predicting DN in patients with DM.

Table 3. Pearson's correlation of α-klotho and Periostin levels with different study parameters

Variables	TSH		T3		T4		Melatonin	
	r	p-value	r	p-value	r	p-value	r	p-value
Age, years	-0.068	0.469	0.001	0.988	-0.008	0.952	-0.116	0.220
Weight, kg	-0.069	0.468	0.010	0.920	-0.022	0.814	0.009	0.347
Height, cm	-0.085	0.368	0.165	0.080	0.058	0.538	0.027	0.771
BMI, kg/m^2	-0.099	0.294	-0.052	0.585	0.017	0.858	0.090	0.340
FBS, mg/dl	0.046	0.629	0.075	0.430	0.008	0.933	0.010	0.919
HbA1c, %	-0.050	0.537	-0.013	0.888	0.060	0.472	0.055	0.561
HD Duration	-0.112	0.392	0.013	0.923	-0.075	0.570	0.026	0.843
TSH			**-0.544**	**<0.001**	**-0.569**	**<0.001**	0.118	0.215
T3					**0.776**	**<0.001**	-0.016	0.869
T4							-0.088	0.354

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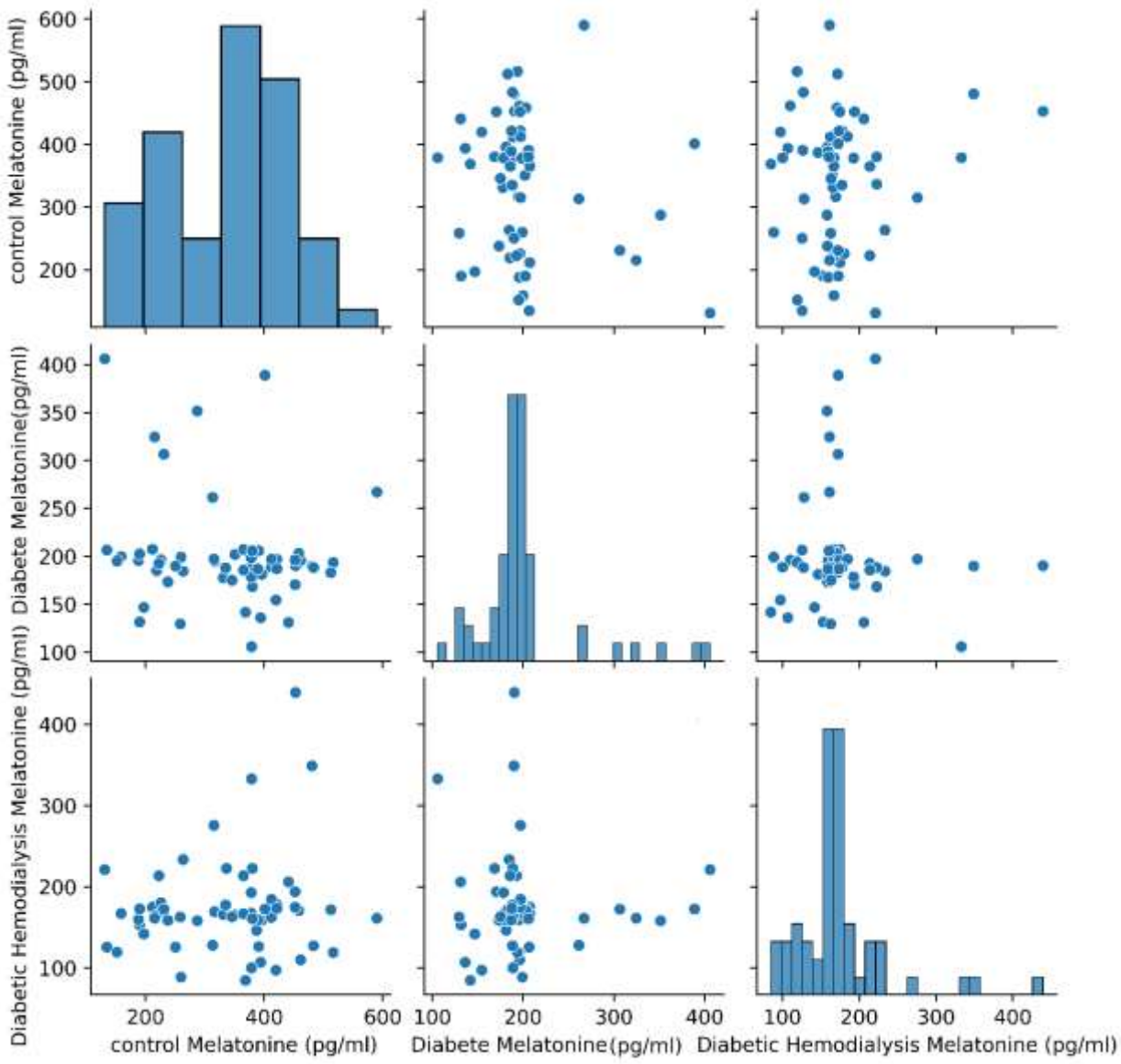

Fig. 3. The pair plot analysis is used to analyze the relationships between different variables pairwise. Each scatter plot in this pair plot represents the relationship between two variables, while the diagonal plots show the distribution of each variable.

Discussion

According to this research, thyroid function tests showed no significant variation between diabetic patients with and without DN. In a Chinese study, Zhao et al. (13) enrolled one hundred T2DM patients without DN and one hundred thirty-nine with DN. Subclinical hypothyroidism (SCH) and low FT3 levels were more frequent in patients with T2DM than DN (20.9% vs. 10.8%) ($p < 0.05$). Furthermore, in patients with DN, there were positive correlations between TSH and serum creatinine ($r = 0.363$, $p = 0.013$), which contradicts our findings. In another study, Liu et al. (14) found that FT3 could predict the progression of renal impairment in patients with T2DM. This discrepancy between studies may be related to variations in study design and participant characteristics, such as sex and age composition. Previous studies have found that the female and elderly populations are more frequently affected by SCH (15). Furthermore, the latter two studies measured FT3, which is more accurate than T3, which was used in our research.

Thyroid hormones can impact renal growth, glomerular and tubular function, and

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renal hemodynamics and activate the renin-angiotensin-aldosterone system (16). They may also influence renal function through cardiovascular and systemic hemodynamic effects in addition to acting directly on the kidney (17).

The most exciting finding in the present study was that melatonin levels were significantly reduced in DN, and melatonin was a good predictor for the progression of DN in patients with T2DM. To our knowledge, this is the first research to address the predictive value of melatonin for DN. On the other hand, several previous studies have emphasized the role of melatonin in protecting patients from T2DM or DN in those patients. McMullan et al. (18) have demonstrated that decreased melatonin secretion was independently associated with an increased risk of developing type 2 diabetes. Motawi et al. (19) argued that melatonin decreases the impact of T2DM on oxidative stress biomarkers. Furthermore, melatonin prevents endothelial-to-mesenchymal transition (EMT) in glomerular endothelial cells exposed to transforming growth factor-β2 (TGF-β2) and in the glomeruli of diabetic rats; this effect is mediated by the up-regulation of miR-497 expression, leading to suppression of RhoA/Rho kinase (ROCK) activity (20).

Onk et al. (21) found that the administration of melatonin in experimentally diabetic rats significantly reduced kidney tissue levels of inflammatory cytokines, oxidative stress biomarkers, and IL-33, suggesting the potential roles of melatonin in managing diabetic nephropathy. Additionally, melatonin has been shown to replenish the decreased levels of nitric oxide, glutathione peroxidase, and superoxide dismutase in diabetes (22).

Based on the results of the present and previous studies, it is reasonable to assume that factors associated with lower melatonin secretion, such as sleep disturbance and snoring, could exacerbate the risk of DN progression in patients with T2DM and increase the risk of T2DM in healthy individuals.

Collectively, these data indicate that thyroid hormones have little or no impact on the progression of DN in patients with T2DM. In contrast, melatonin levels are significantly reduced in patients with DN and could be routinely used as an indicator of the progression of this complication. Clinically, physicians should recommend to their diabetic patients that they ensure sufficient sleep duration and eliminate factors that disrupt sleep to maintain adequate melatonin levels, which may reduce the incidence of T2DM complications.

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Conflict of Interest

No conflict of interest.

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