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## Correlation between thyroid hormone values and anemia in elderly patients with diabetic nephropathy.

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**Keywords:** correlation; diabetic nephropathy; elderly; anemia severity; thyroid hormone.

**Abstract.** We aimed to explore the correlation between thyroid hormone levels and anemia severity in elderly diabetic nephropathy (DN) patients. Elderly DN patients (140 in total) diagnosed and treated during November 2019 and December 2023 were retrospectively recruited as a DN group, 140 patients with uncomplicated diabetes mellitus as a simple diabetes group, and 140 healthy subjects as a healthy group. A non-anemia group (n=63) and an anemia group (n=77) were set up as sets of the DN group according to the hemoglobin (Hb) level, and the anemia group was further divided into a severe group (n=48) (Hb<60 g/L), a moderate group (n=16) (60 g/L≤Hb<90 g/L) and a mild group (n=13) (Hb≥90 g/L). Compared to the simple diabetes group, significantly increased levels of serum TSH and significantly decreased levels of FT4 and FT3 were found in the DN group (p<0.05). A significant increase in TSH levels and significant decreases in FT4 and FT3 levels were detected in the serum from the moderate group compared with those from the mild group (p<0.05). The same trends in these levels were observed from the severe group compared to the moderate group (p<0.05). Hb had a negative correlation with TSH and positive correlations with FT4 and FT3 (p<0.05). High TSH and low FT4 and FT3 may be related to anemia in elderly patients with DN, and they have correlations with the severity of anemia.

## Correlación entre los valores de hormonas tiroideas y anemia en pacientes ancianos con nefropatía diabética.

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**Palabras clave:** correlación; nefropatía diabética; ancianos; gravedad de la anemia; hormona tiroidea.

**Resumen.** Nuestro objetivo fue explorar la correlación entre el nivel de hormona tiroidea y la severidad de la anemia en pacientes ancianos con nefropatía diabética (ND). Se reclutaron retrospectivamente 140 pacientes ancianos con ND diagnosticados y tratados entre noviembre de 2019 y diciembre de 2023, 140 pacientes con diabetes mellitus sin nefropatía y 140 sujetos sanos como grupo control sano. El grupo de ND se dividió en un grupo sin anemia (n=63) y un grupo con anemia (n=77) de acuerdo con el nivel de hemoglobina (Hb). El grupo con anemia fue subdividido en grave (n=48) (Hb<60 g/L), moderada (n=16) (60 g/L-Hb<90 g/L) y leve (n=13) (Hb-90 g/L). En comparación con el grupo de diabetes simple, se encontró un aumento significativo de los niveles séricos de TSH y una disminución significativa de los niveles de FT4 y FT3 en el grupo de DN (p<0,05). Se encontró un aumento significativo del nivel de TSH junto con disminuciones significativas de los niveles de FT4 y FT3 en el suero del grupo moderado en comparación con los del grupo leve (p<0,05). Las mismas tendencias en estos niveles se observaron en el grupo grave cuando se compararon con el grupo moderado (p<0,05). La Hb tuvo una correlación negativa con TSH y correlación positiva con FT4 y FT3 (p<0,05). Niveles altos de TSH y bajos de FT4 y FT3 podrían estar relacionados con anemia y su gravedad en pacientes ancianos con ND.

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### INTRODUCTION

With the development of population aging, the incidence of diabetes mellitus and other chronic diseases has been increasing year by year. It is estimated that diabetes mellitus may affect 642 million people globally by 2040, with an incidence rate of 8.8-10.4%<sup>1</sup>. Being a microvascular complication of diabetes mellitus characterized by the highest prevalence and severity, diabetic nephropathy (DN) has become the leading contributor to end-stage renal disease<sup>2</sup>. As the disease progresses, the patient's renal function gradually declines, decreasing erythropoietin (EPO) produced by the kidney, iron absorption, and

red blood cell survival time, thus significantly increasing the incidence of anemia<sup>3</sup>. Anemia suggests that DN has progressed to a severe stage, and renal failure occurs, resulting in ineffective excretion of metabolites and toxins in the body and further worsening the condition of the disease. In addition, anemia can lead to insufficient oxygen supply to organs, aggravating complications of diabetes mellitus, such as cardiovascular diseases and retinopathy. Diabetic patients with chronic anemia are at higher risk of developing cardio-cerebrovascular diseases, retinopathy, nephropathy and neuropathy.

In addition, due to a significant decrease in urine protein and gradual impact

on the hypothalamic-pituitary-thyroid axis function with the progression of the disease, the synthesis of thyroid hormones declines in DN patients, and thyroid dysfunction occurs. Thyroid hormones may be implicated in glomerular filtration rate (GFR) regulation and blood circulation in the kidneys<sup>4</sup>. The rate of thyroid function abnormality in patients with kidney disease at stage G5 is significantly higher than that at stage G1 (39.1% vs. 8.3%), and it is considered that the severity of kidney disease may be closely related to thyroid function<sup>5</sup>. Moreover, the severity of type 2 diabetes mellitus (T2DM) in elderly patients also correlates with blood glucose-related indicators and thyroid hormone levels. Free triiodothyronine (FT3), free thyroxine (FT4) and other thyroid hormones in mild and severe T2DM in elderly patients are at significantly lower levels than in healthy controls<sup>6</sup>, suggesting that the changes in thyroid hormone levels may be related to the severity of T2DM in elderly patients. However, no reports are available yet on whether thyroid hormone level is related to the severity of anemia in elderly DN patients.

Because of this, the level changes of thyroid hormones in patients with different severities of anemia were analyzed in this study to explore the correlations of thyroid hormones with anemia severity in elderly DN subjects, aiming to provide a reference for diagnosis and treatment in clinical practice.

## PATIENTS AND METHODS

### Subjects

One hundred and forty older adults diagnosed with DN and hospitalized for treatment herein during November 2019 and December 2023 were retrospectively recruited into the DN group. The following inclusion criteria were utilized: 1) patients satisfying the diagnosis and classification criteria for diabetes mellitus in the *Guideline for Prevention and Treatment of Type 2 Diabetes Mellitus in China* (2020 Edition)<sup>7</sup>, 2) those

who met the diagnostic and treatment criteria for DN<sup>8</sup>, 3) those with urinary albumin excretion rate (AER)  $\geq 30$  mg/24 h,  $GFR \leq 60$  mL/min $\cdot 1.73$  m<sup>2</sup> or urinary microalbumin/creatinine ratio (ACR)  $\geq 3$  mg/g, and 4) those without renal transplantation or dialysis history. The adopted exclusion criteria included: 1) patients who had taken drugs that may affect urinary protein excretion before participating in the study, 2) those with no obvious hepatic and renal dysfunction previously and no severe complications of diabetes mellitus recently, 3) those complicated with other kidney diseases, or 4) those complicated with mental illness or cognitive dysfunction. Another 140 patients with uncomplicated diabetes mellitus entered the simple diabetes group, in addition to 140 healthy subjects as the healthy group. Their gender, age, and body mass index were not significantly different from those in the DN group.

### Grouping criteria

According to the GFR, patients with chronic DN were divided into stage 5 group (n=11,  $GFR < 15$  mL/min $\cdot 1.73$  m<sup>2</sup>), stage 4 group (n=20,  $15$  mL/min $\cdot 1.73$  m<sup>2</sup>  $\leq GFR < 30$  mL/min $\cdot 1.73$  m<sup>2</sup>), stage 3 group (n=43,  $30$  mL/min $\cdot 1.73$  m<sup>2</sup>  $\leq GFR < 60$  mL/min $\cdot 1.73$  m<sup>2</sup>), stage 2 group (n=39,  $60$  mL/min $\cdot 1.73$  m<sup>2</sup>  $\leq GFR < 90$  mL/min $\cdot 1.73$  m<sup>2</sup>), and stage 1 group (n=27,  $GFR \geq 90$  mL/min $\cdot 1.73$  m<sup>2</sup>).

An anemia group (n=77) plus a non-anemia group (n=63) were established as subsets of the DN group according to the hemoglobin (Hb) level.  $Hb \leq 120$  g/L in females and  $\leq 130$  g/L in males indicated anemia.

The anemia group was further divided into a severe group (n=48) ( $Hb < 60$  g/L), a moderate group (n=16) ( $60$  g/L  $\leq Hb < 90$  g/L) and a mild group (n=13) ( $Hb \geq 90$  g/L).

### Detection of thyroid hormones

All patients were enrolled to collect fasting venous blood (5 mL) in the morning for centrifugation. Then, the supernatant was

harvested to measure thyroid-stimulating hormone (TSH), FT4 and FT3 using an MPI-A electrochemiluminescence analyzer (Xi'an Remex Analysis Instruments Co., Ltd.) in strict accordance with the kit instructions. TSH (Item No.: ZY-TSH-Hu), FT4 (Item No.: ZY-FT4-Ge) and FT3 test kits (Item No.: ZY-FT3-Ge) were purchased from Shanghai Zeye Biotechnology Co., Ltd.

### Statistical analysis

The SPSS 24.0 software for statistical analysis was employed. The description format of ( $\bar{x} \pm SD$ ) was utilized for the measurement data distributed normally, which were compared by the independent-sample *t*-test between two groups and by the univariate multi-sample mean test among multiple groups. The count data were described by [n (%)] and subjected to the chi-square test for comparisons. The correlations of thyroid hormone level with anemia severity were explored by the Pearson analysis. A difference with statistical significance was marked with  $p < 0.05$ .

## RESULTS

### Baseline data

Table 1 exhibits the three groups of baseline data.

### Thyroid hormone levels in DN patients

Compared to the healthy group, a significant increase in TSH levels and significant decreases in FT4 and FT3 levels were detected in the serum from the simple diabetes group ( $t=19.307, -6.34, -16.16; p < 0.05$ ). In comparison with the simple diabetes group, significantly increased levels of serum TSH and significantly decreased levels of FT4 and FT3 were observed in the DN group ( $t=17.989, -6.702, -14.076; p < 0.05$ ) (Table 2).

### Association between DN and anemia

In the DN group, anemia occurred in 77 cases, including 7, 11, 32, 16 and 11 cases in stages 1-5, respectively. The incidence and severity of anemia were found to rise gradually as the stage of DN increased ( $p < 0.05$ ) (Table 3).

### Changes in thyroid hormone levels in patients with different severities of anemia

The serum levels of TSH, FT4 and FT3 in the severe, moderate, mild, and non-anemia groups were detected with significant differences ( $p < 0.05$ ). Compared to the mild group, the moderate group exhibited evident elevations in serum TSH levels and marked decreases in FT4 and FT3 levels ( $t=2.613,$

Table 1. Baseline data.

Group	n	Sex n (%)		Age (years) $\bar{x} \pm SD$	Body mass index (kg/m <sup>2</sup> ) $\bar{x} \pm SD$	Smoking history n (%)	Drinking history n (%)
		Female	Male				
DN	140	77 (55)	63 (45)	52.84±8.15	23.69±2.26	81 (57.86)	87 (62.14)
Simple diabetes	140	71 (50.71)	69 (49.29)	53.15±7.26	24.12±2.24	76 (54.29)	80 (57.14)
Healthy	140	74 (52.86)	66 (47.14)	52.77±7.49	24.08±2.32	75 (53.57)	82 (58.57)
<i>F/χ</i> <sup>2</sup>		0.516		0.10	1.53	0.597	0.769
<i>p</i>		0.773		0.907	0.218	0.742	0.681

DN: Diabetic nephropathy. The measurement data were compared by univariate multi-sample mean test among multiple groups. The count data were subjected to the chi-square test for comparisons.

**Table 2.** Thyroid hormone levels in diabetic nephropathy patients.

Group	n	TSH ( $\mu$ IU/mL) $\bar{X} \pm SD$	FT4 (pmol/L) $\bar{X} \pm SD$	FT3 (pmol/L) $\bar{X} \pm SD$
DN	140	5.83 $\pm$ 1.72 <sup>ab</sup>	13.75 $\pm$ 4.01 <sup>ab</sup>	3.55 $\pm$ 0.93 <sup>ab</sup>
Simple diabetes	140	2.85 $\pm$ 0.94 <sup>a</sup>	17.31 $\pm$ 4.84 <sup>a</sup>	5.51 $\pm$ 1.36 <sup>a</sup>
Healthy	140	1.17 $\pm$ 0.42	20.79 $\pm$ 4.33	9.70 $\pm$ 2.75
F		582.14	89.34	403.37
p		<0.001	<0.001	<0.001

The measurement data were compared by univariate multi-sample mean test among multiple groups. <sup>a</sup>p<0.05 vs. healthy group, <sup>b</sup>p<0.05 vs. simple diabetes group.

**Table 3.** Association between diabetic nephropathy and incidence and severity of anemia.

Group	n	Severe n (%)	Moderate n (%)	Mild n (%)	None n (%)
Stage 5	11	9 (81.82)	1 (9.09)	1 (9.09)	0 (0)
Stage 4	20	11 (55)	4 (20)	1 (5)	4 (20)
Stage 3	43	24 (55.81)	5 (11.63)	3 (6.98)	11 (25.58)
Stage 2	39	4 (10.26)	3 (7.69)	4 (10.26)	28 (71.79)
Stage 1	27	0 (0)	3 (11.11)	4 (14.81)	20 (74.07)
$\chi^2$					57.405
p					<0.001

The count data were subjected to the chi-square test for comparisons.

-1.88, -3.439; p<0.05). The TSH levels climbed significantly, whereas the FT4 and FT3 levels dropped significantly in the serum from the severe group when compared with the moderate group (t=3.555, -4.598, 6.366; p<0.05) (Table 4).

#### Correlation of thyroid hormone levels with anemia severity

As revealed by the Pearson's correlation analysis, Hb had a negative correlation with TSH, but it had a positive correlation with FT4 and FT3 (p<0.05) (Fig. 1).

### DISCUSSION

DN has the early pathological characteristics of glomerular basement membrane thickening and mesangial expansion and the late characteristics of glomerulosclerosis and renal tubule interstitial fibrosis<sup>9</sup>.

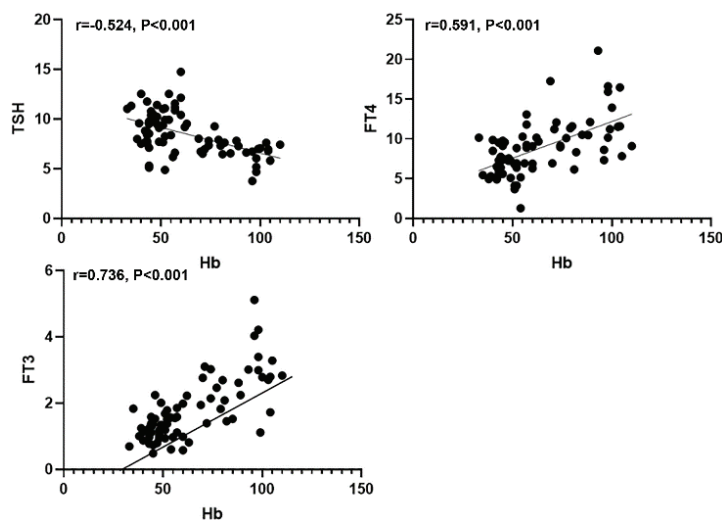
Early diagnosis and intervention are crucial for managing DN, particularly in elderly patients, who are often more vulnerable to its progression due to poor diabetes management and comorbidities. Therefore, predicting disease progression and achieving timely intervention in elderly DN patients are vital for improving the outcomes.

A growing body of research has highlighted the correlation between thyroid dysfunction and DN. Hypothyroidism, a common thyroid disorder in patients with T2DM, has been identified as a potentially significant contributing factor to DN<sup>10,11</sup>. Thyroid hormones play a critical role in regulating metabolic processes. These hormones contribute to the development of the body, support cardiac output, and promote myocardial contractility. Additionally, they are involved in maintaining renal function stability<sup>12</sup>.

**Table 4.** Changes in thyroid hormone levels in diabetic nephropathy subjects with various severities of anemia and no anemia.

Group	n	TSH ( $\mu$ IU/mL) $\bar{x} \pm SD$	FT4 (pmol/L) $\bar{x} \pm SD$	FT3 (pmol/L) $\bar{x} \pm SD$
Non-anemia	63	3.30 $\pm$ 0.76	15.17 $\pm$ 2.30	4.29 $\pm$ 1.02
Anemia				
Mild	13	6.28 $\pm$ 1.19 <sup>a</sup>	12.42 $\pm$ 3.15 <sup>a</sup>	3.07 $\pm$ 0.86 <sup>a</sup>
Moderate	16	7.64 $\pm$ 1.61 <sup>ab</sup>	10.37 $\pm$ 2.61 <sup>ab</sup>	2.14 $\pm$ 0.51 <sup>ab</sup>
Severe	48	9.41 $\pm$ 2.03 <sup>abc</sup>	7.16 $\pm$ 1.72 <sup>abc</sup>	1.26 $\pm$ 0.37 <sup>abc</sup>
F		168.00	117.38	141.03
p		<0.001	<0.001	<0.001

The measurement data were compared by univariate multi-sample mean test among multiple groups. <sup>a</sup>p<0.05 vs. non-anemia group, <sup>b</sup>p<0.05 vs. mild group, <sup>c</sup><0.05 vs. moderate group.

**Fig. 1.** Correlation between thyroid hormones and anemia severity.

Hypothyroidism may exacerbate DN through several mechanisms. One of the most critical effects is the reduction in insulin sensitivity, which leads to worsened blood glucose control and insulin resistance. This dysfunction is compounded by the inflammatory response triggered by hyperglycemia, which inhibits 5'-deiodinase activity, a key enzyme involved in converting T4 to T3. This reduction in FT3 levels damages the renal tubular function and reduces the renal plasma flow and glomerular capillary hydrostatic pressure, further compromising renal function<sup>13</sup>. In addition, DN-associated hyperglycemia and other metabolic disturbances

can directly or indirectly affect the hypothalamic-pituitary-thyroid axis, impairing thyroid hormone production and contributing to worsening renal function.

In this study, DN patients had significantly increased levels of serum TSH and decreased levels of FT3 and FT4, indicating that thyroid hormone dysregulation may be an important factor in the progression of DN. As kidney function declines, the ability of the kidney to convert T4 to T3 through 5'-deiodinase activity is diminished. Additionally, metabolic disturbances in DN, including selenium deficiency (a cofactor for 5'-deiodinase), further exacerbate the dys-



function of thyroid hormone conversion<sup>14</sup>. As the disease progresses, T3 levels continue to decrease, and this reduction is correlated with worsening renal function<sup>15</sup>. Furthermore, serum T3 levels have been shown to serve as an independent risk factor for DN, with lower T3 levels associated with greater disease severity<sup>16,17</sup>.

Another important aspect of DN progression is anemia, which frequently occurs as a complication and significantly affects patient outcomes<sup>18</sup>. Anemia in DN is primarily caused by reduced EPO production as kidney function deteriorates. EPO, which is secreted by the kidneys, plays a central role in red blood cell production<sup>19</sup>. As kidney function declines, EPO secretion is impaired, leading to anemia. The incidence of anemia in DN can reach 100% in stage 5 DN, and its severity is correlated with the degree of renal dysfunction<sup>20</sup>. Consistently, we found in this study that the anemia severity was closely related to the progression of DN, with Hb levels as an important marker for disease severity.

In addition to kidney dysfunction, thyroid hormone levels also influence anemia. Disruption of thyroid hormone function may exacerbate anemia in DN patients through various mechanisms. Specifically, thyroid hormone resistance can disrupt the balance between erythropoiesis and the differentiation of erythrocyte progenitor cells. Besides, chronic anemia can lead to altered endocrine function and impaired EPO secretion<sup>21</sup>. In the present study, the serum TSH levels increased significantly, and the levels of FT4 and FT3 decreased with increasing severity of anemia. All of these levels had significant associations with Hb. Hence, the impaired kidney function in DN reduces the kidney's ability to regulate thyroid hormones, contributing to further disturbances in thyroid function<sup>22</sup>.

Furthermore, anemia itself may induce a stress state, thus affecting the hypothalamic-pituitary-thyroid axis function. Under stress, more thyrotropin-releasing hormone

may be secreted from the hypothalamus to promote the secretion of more TSH by the pituitary gland. However, due to renal dysfunction and the loss of thyroid hormone-binding proteins, the synthesis and secretion of thyroid hormones remain impaired, resulting in decreased levels of FT4 and FT3<sup>23</sup>. This cycle may aggravate the anemia and accelerate the progression of DN.

Exploring the relationship between thyroid hormone disruption and anemia severity in elderly DN patients provides important insights for clinical management. By monitoring thyroid hormone levels besides kidney function and anemia status, healthcare providers may be able to predict better disease progression and tailor interventions to mitigate adverse outcomes. Our study contributes to this understanding by demonstrating that thyroid hormone imbalances, particularly low FT3 and FT4 levels, are closely associated with the severity of anemia in DN patients.

Nevertheless, this study has limitations. The duration of this study was short, the sample size was small, and no data were collected on the association between the severity of anemia and thyroid hormone levels in patients undergoing kidney transplantation or dialysis. Therefore, further studies are still required.

In conclusion, high TSH and low FT4 and FT3 may be the factors related to anemia in elderly patients with DN, and they have associations with the severity of anemia.

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#### Conflict of Interest

The authors declare no conflict of interest.

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All authors had participated in the development and writing of the paper.

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