Estimating the Biological Variables of Thyroid Patients and Their Impact on Diabetics in Kirkuk

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ABSTRACT
Background: Investigating the impact of sugar on biochemical variables in patients with thyroid dysfunction in Kirkuk, including some previously unmeasured biological variables. Materials and Techniques: From November to May, this study occurred in Kirkuk. Blood samples were collected from 120 patients aged 20 to 80 years, including 40 diabetics, 40 with both hyperthyroidism and diabetes, and 40 with both hypothyroidism and diabetes, as well as 40 healthy individuals aged 20 to 40 without chronic diseases. Levels of TSH, T4, T3, HbA1c, GPT, GOT, ALP, and HBs were measured in the serum using Beckman Coulter Access2, Spectrophotometer, and Boditech Med INC I-Chroma AFP-25 devices. Results: The study found that TSH levels were significantly elevated in patients with diabetes and hypothyroidism ($P \leq 0.01$), while T4 and T3 levels were significantly decreased ($P \geq 0.01$). HbA1c levels showed a significant increase ($P \leq 0.01$) in all groups: diabetes with hypothyroidism, diabetes with hyperthyroidism, and diabetes alone. Enzyme levels (GOT, GPT, ALP, HBs) were also significantly elevated ($P \leq 0.01$) in these groups. Conclusion: In conclusion, TSH regulation is vital, with high levels indicating hypothyroidism related to obesity and diabetes. Elevated HbA1c shows poor glucose control, and hyperthyroidism with diabetes worsens liver function and glucose metabolism, stressing the need for integrated management.

1. Introduction

Diabetes (DM) is a group of diseases characterized by high levels of glucose in the blood, resulting from defects in the body's ability to produce or use insulin. It is marked by elevated blood sugar levels, leading to complications such as damage to microscopic blood vessels, including retinopathy, neuropathy, and kidney disease(1) (2). Chronic high blood sugar results from insufficient insulin production (type 1 diabetes) or inadequate cellular response to insulin (type 2 diabetes). Diabetes may present with symptoms such as thirst, frequent urination, blurred vision, and weight loss, though these symptoms can sometimes be mild or absent(3) . The condition is often caused by an imbalance in insulin secretion from the pancreas, where either insulin production is insufficient or there is a complete lack of insulin, known as insulin deficiency(4). Diabetes is a chronic, progressive disease characterized by persistently high blood glucose levels(5). Risk factors for type 2 diabetes (T2DM) include obesity, an unhealthy diet, lack of physical activity, increasing age, insulin resistance, family history of diabetes, genetic factors, and race (6). Thyroid disease negatively impacts diabetes control and is commonly associated with autoimmune diseases in type 1 diabetes (T1DM) and advanced age in type 2 diabetes(7). The interplay between diabetes and thyroid disorders is well-documented. Thyroid hormones help regulate pancreatic function and carbohydrate metabolism, while diabetes can affect thyroid function tests to varying degrees (8). Thyroid disorders can increase the risk of diabetes and complicate blood sugar control. Important nerves move through the gland known as the thyroid, which is the biggest reproductive gland in the body, and is located at the front of the neck above the Adam's apple. (9). Thyroid hormones are essential for maintaining tissue metabolism, stimulating oxygen consumption, and regulating fat and carbohydrate metabolism(10). Hypothyroidism and hyperthyroidism are the most common autoimmune thyroid disorders and are significant complications of thyroid dysfunction(11).

2. Material and Procedures

Study Design, Participants, and Sample Gathering.

120 participants were recruited for the study, 40 of whom had diabetes and hypothyroidism, 40 of

whom had diabetes and hyperthyroidism, and 40 of whom had diabetes alone., aged 20 to 70 years. The patients were from Azadi Teaching Hospital, Kirkuk General Hospital, and Hawija General Hospital, and included both sexes. After obtaining written consent, 5 ml of venous blood was drawn from each participant using a sterile syringe and transferred to laboratory tubes. One tube contained ethylene diamine tetraacetic acid (EDTA), and two tubes were left empty. Blood in the EDTA tube was used for determining cumulative sugar levels using immunofluorescence technology. The remaining blood was added to other laboratory tubes for serum extraction. Following a 10-minute centrifugation at 3000 rpm on the samples, the serum was separated and put into Eppendorf tubes. These tubes were stored at -20°C for measuring triiodothyronine, thyroxine, thyroid-stimulating hormone, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and viral hepatitis markers.

Determination of serum TSH, T4 , T3 level.

The Beckman Coulter Access2 instrument was used to determine the blood serum's TSH level., which operates on an immunofluorescence assay system. This system detects the interaction between antibodies and antigens to measure specific variables in the blood serum. The device uses a reagent package containing manufactured antibodies and a conjugation solution with a substrate solution. The washing solution, available inside the device, is used for all measurements, guided by the barcode on the detector which contains the necessary information. Serum samples are added to the device's test tubes, and the type of test to be performed is selected via the device screen. The measurement process then proceeds as follows:

The device first withdraws serum from the test tubes and the antibody and conjugation solutions from the reagent package, adding them to a reaction vessel where they are mixed thoroughly with a special pipette. The reaction vessel is then incubated to allow the antibody to bind with the antigen and conjugation solution. Next, the reaction vessel is washed three times with the washing solution in a magnetic field to remove any unrelated substances. A substrate solution is added to the reaction vessel, which binds with the conjugate, generating a glowing light. The device’s reader measures the fluorescence from this reaction, converts it to concentrations using a pre-entered calibration curve, and displays the results with a laboratory number on the device’s screen.

Determination of (HbA1c) level.

Procedure

To perform the test, first draw 100 µL from the hemolysis solution container and transfer it to the reagent solution tube. Next, add 5 µL of blood to the same tube, close the cap, and mix the sample thoroughly by shaking it 10 times. Transfer 75 µL of this mixture to the test slide and incubate it for 12 minutes. After incubation, place the slide into the holder of the device, where the test strip is scanned. Finally, read the results on the device's display screen.

Measuring the activity of the enzyme Aspartate amino transferase in serum.

An aspartate aminotransferase (AST) enzyme activity assay was conducted with a diagnostic kit manufactured by Randox Company (United Kingdom). The enzyme activity was measured by assessing the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine, which produces a brown color. This color intensity, proportional to the amount of enzyme in the serum, was measured using a spectrophotometer at a wavelength of 546 nm (Reitman and Frankel, 1957).

Measurement of the effectiveness of the Alanine Aminotransferase in the serum

An aspartate aminotransferase enzyme diagnostic kit made by Randox (UK) was used to measure the enzyme's activity. By measuring the concentration of pyruvate hydrazone produced using 2,4-dinitrophenylhydrazine, which creates a brown hue detectable by a spectrophotometer at a wavelength of 546 nm, the activity of the ALT enzyme was determined. According to Reitman

and Frankel (1957), the intensity of color corresponds to the concentration of enzyme in the serum.

Measurement of the activity of Alkaline phosphatase enzyme in serum.

The color intensity of alkaline phosphatase enzyme activity was measured, and the liberated phenol was assessed in the presence of 4-aminoantipyrine and potassium ferricyanide. The enzymatic reaction is stopped by sodium arsenate in the detector, resulting in a brown color that can be measured at 510 nm with a spectrophotometer. The amount of enzyme in the serum is directly correlated with the color intensity. The ingredients were combined and let sit in a dark area for ten minutes, after which the absorbance of the sample and the standard was read against their blanks using a spectrophotometer at 510 nm. The color of the reaction remained stable for 45 minutes

Determination of serum Hepatitis B level

The sandwich immunoassay method is used in this test. A second antibody that has been immobilized on the test strip captures the antigen-antibody complex that is formed when the dried detector attaches to the antigen in the sample and migrates to the nitrocellulose matrix. The amount of antigen in the sample increases the formation of antigen-antibody complexes, which intensifies the fluorescence signal on the detecting equipment.

Analytical Statistics

GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA) was used for the statistical analysis, and the data are presented as mean ± standard deviation (SD). The ANOVA test was used to compare the means, and $P \leq 0.05$ was considered statistically significant. Also examined was the parameter correlation**.**

3. Results and Discussion

Thyroid Stimulating Hormone (TSH)

Table 1 displays the findings of the current study. The concentration of thyroid-stimulating hormone (TSH) was found to be significantly higher ($P \leq 0.01$) in the group with hypothyroidism and diabetes (4.43 ± 18.15 microunits/ml) than in the groups with hyperthyroidism and diabetes (0.07 ± 0.15 microunits/ml), diabetes alone (0.90 ± 2.40 microunits/ml), and control (0.75 ± 2.30 microunits/ml). In comparison to the diabetes-only and control groups, the study also found a statistically significant drop in TSH levels in the group with diabetes and hyperthyroidism. Nonetheless, there were no appreciable variations in TSH levels between the control and Type 2 diabetes subgroups.

Thyroxin (T4)

The current study's results, displayed in Table 1, demonstrate a significant increase ($P \leq 0.01$) in the thyroxine hormone T4 concentration in the group with hyperthyroidism and diabetes (34.86±21.20 nmol/L) as compared to the groups with hypothyroidism and diabetes (20.28±5.22 nmol/L), diabetes alone (18.78±11.9 nmol/L), and the control group (22.63±13.7 nmol/L). In addition, compared to the control group and those with diabetes alone, the group with diabetes plus hypothyroidism showed a statistically significant drop in T4 concentration. Nevertheless, there were no appreciable variations in T4 concentration between the control group and the diabetic group.

Triiodothyronine

According to Table 1's results, the group of people with diabetes and hyperthyroidism had a significantly higher concentration of the hormone triiodothyronine (1.06 ± 3.24 nmol/L) than the groups with diabetes and hypothyroidism (0.23 ± 0.89 nmol/L), the group with diabetes alone (0.30 ± 1.38 nmol/L), and the control group (0.44 ± 1.36 nmol/L). Comparing the group with

diabetes and hypothyroidism to the groups with diabetes alone and the control group, the study likewise found a substantial drop in T3 levels. But there were no appreciable variations in T3 concentration between the control group and the diabetes group.

Table (1): Levels of biochemical variables in the serum of patients with diabetes, diabetes with hyperthyroidism, and diabetes with hypothyroidism compared to a control group.

parameters	Mean ± SD Control	Mean ± SD Diabetes	Mean ± SD Diabetes hypothyrodism	Mean ± SD Diabetes hyperthyrodism
TSH (µIU/ml)	2.308 ± 0.75	2.405 ± 0.908	18.15 ± 4.43[a,b]	0.151± 0.07[c]
T4 (nmol/L)	113.7 ± 22.63	111.9±18.78	65.22 ± 20.28[a,b]	218.2±34.86[a,b,c]
T3 (nmol/L)	1.369 ± 0.44	1.388 ± 0.302	0.895 ± 0.23[a,b]	3.24 ± 1.06[a,b,c]

Where: (a) significant with control, (b) significant with diabetes, (c) significant with hypothyrodism.

Hemoglobin A1c (HbA1c)

In comparison to the control group (0.67±5.13), the cumulative glucose concentrations in the groups with diabetes and hypothyroidism (2.03±9.71), diabetes and hyperthyroidism (2.02±10.78), and diabetes alone (1.02±10.03) significantly increased ($P \leq 0.01$) in the current study's results (Table 2). Between the group with diabetes and hyperthyroidism and the group with diabetes alone, there were no discernible variations in the cumulative glucose concentration.

Table (2): HbA1c levels in the blood of patients with diabetes, diabetes with hyperthyroidism, and diabetes with hypothyroidism compared to a healthy group.

parameters	Mean ± SD Control	Mean ± SD Diabetes	Mean ± SD Diabetes hypothyrodism	Mean ± SD Diabetes hyperthyrodism
HbA1C (%)	5.134 ± 0.675	10.03 ± 1.021[a]	9.715 ± 2.021[a]	10.78 ± 2.021[a,c]

Where: (a) significant with control, (b) significant with diabetes, (c) significant with hypothyrodism.

Aspartate aminotransferase (AST)

The current study's results (Table 3) show that the concentration of the enzyme aspartate aminotransferase was significantly higher in the diabetes group (10.22 ± 40.51 IU/L) than in the thyroid insufficiency and hyperthyroidism groups (10.94 ± 30.86 IU/L and 8.63 ± 31.94 IU/L), relative to the control group (6.27 ± 21.44 IU/L). The group with diabetes alone had the greatest value. Additionally, there were no discernible variations in GOT concentration between the groups with diabetes and hypothyroidism and hyperthyroidism, according to the results..

Alanine Aminotransferase (ALT)

According to the results of the current study, there was a significant increase ($P \leq 0.01$) in the concentration of the enzyme alanine aminotransferase (ALT) in the group of people with diabetes (9.56 ± 37.98 IU/L) compared to the group with diabetes and hypothyroidism (0.38 ± 32.36 IU/L) and the group with diabetes and hyperthyroidism (8.07 ± 30.27 IU/L). Both of these groups had higher ALT levels compared to the control group (5.29 ± 21.44 IU/L). The highest value was recorded in the diabetes-only group. The study also found no significant differences in ALT concentration between the groups with diabetes and hyperthyroidism and those with diabetes and hypothyroidism.

Alkaline phosphatase (ALP)

The concentration of the enzyme alkaline phosphatase (ALP) was found to be significantly higher ($P \leq 0.01$) in the group of individuals with diabetes (35.54±145.30 IU/L) than in the groups with diabetes and hypothyroidism (22.37±88.28 IU/L) and hyperthyroidism (24.0±98.16 IU/L), as well as in the control group (15.16±62.97 IU/L). The group with diabetes alone had the highest ALP value. Between the groups with diabetes and hyperthyroidism and the groups with diabetes and hypothyroidism, there were no discernible variations in ALP concentrations.

Hepatitis B surface (HBV)

As presented in Table (3), the current study's findings indicate a significant increase ($P \leq 0.01$) in the levels of hepatitis B virus (HBV) in the groups with diabetes (0.32±1.24) and hypothyroidism (0.12±1.11) when compared to the control group (0.07±0.23) and the group with hyperthyroidism (0.14±0.47). The group that just had diabetes had the greatest value. Furthermore, the data showed no discernible variations in HBV levels between the groups with diabetes and hypothyroidism and those with diabetes alone.

Table (3): The concentration of biochemical variables (AST, ALT, ALP, and HBV) in the sera of patients with diabetes, diabetes and hyperthyroidism, and diabetes and hypothyroidism compared to a healthy group.

Parameters	Mean ± SD Control	Mean ± SD Diabetes	Mean ± SD Diabetes hypothyrodism	Mean ± SD Diabetes hyperthyrodism
AST (U/L)	21.70 ± 6.27	40.51 ± 10.22[a]	30.86± 10.94[a]	31.94± 8.63
ALT (U/L)	21.44 ± 5.29	37.98± 9.56[a]	32.36± 8.03	30.27± 8.07[a]
ALP (U/L)	62.97 ± 15.16	145.3 ± 35.54[a]	88.28 ± 22.37[a,b]	98.16 ± 24.0[b]
HBV (IU)	0.239 ± 0.07	1.124 ± 0.321[a]	1.113 ± 0.129	0.479 ± 0.141[a,b,c]

Where: (a) significant with control, (b) significant with diabetes, (c) significant with hypothyrodism.

One of the most important markers of thyroid function is thyroid-stimulating hormone (TSH). Since the pituitary gland tries to stimulate the thyroid gland to create more thyroid hormones (T3 and T4), high TSH levels typically suggest hypothyroidism.(12). TSH is secreted by the anterior pituitary gland under the influence of thyrotropin-releasing hormone from the hypothalamus, which directly stimulates the synthesis of thyroid hormones. Thyroid metabolism is regulated through a negative feedback system in individuals with a functioning hypothalamic-thyroid axis. The pituitary gland controls TSH levels in response to feedback from free thyroid hormones (FT4 and FT3), acting as biosensors of thyroid hormone levels. The pituitary and thyroid are in good health. (13). High TSH levels in patients with hypothyroidism may be due to elevated leptin levels, commonly found in obese individuals (body mass index of 30 kg/m²). Leptin primarily signals fat levels to the brain, reducing appetite and food intake. Obesity increases TSH through central and peripheral mechanisms: elevated leptin and proopiomelanocortin stimulate TRH secretion in the paraventricular nucleus, leading to increased TSH. In hypothyroidism, low T4 and high TSH indicate that the pituitary sends more TSH to stimulate the thyroid, which fails to respond. High TSH levels can also be linked to hypothyroidism, which is known to reduce the half-life of circulating insulin(14) . Diabetes affects thyroid function by altering TSH levels and impeding the conversion of T4 to T3 in peripheral tissues. Diabetic ketoacidosis can decrease T3 and T4 levels while TSH remains normal, leading to insulin resistance and hyperthyroidism. Increased blood insulin can cause thyroid tissue proliferation, nodular thyroid disease, and goiter(15). Increased T4 and T3 production in hyperthyroid and diabetic patients may result from single or multiple thyroid nodules, leading to higher iodide uptake and T4 and T3 production while decreasing TSH secretion. This may be due to excess iodine intake, thyroid inflammation,

or excessive synthetic thyroid hormone(16). Glycated hemoglobin (HbA1c) is the primary biomarker for assessing long-term blood sugar control in diabetics. High HbA1c levels indicate poor glucose control and increased blood viscosity, which affects red blood cell flexibility and increases glucose binding to hemoglobin(17). High HbA1c results from elevated blood glucose levels, leading to non-enzymatic glucose addition to hemoglobin, proportional to the age of red blood cells, making it a good indicator of long-term high blood sugar(18). HbA1c formation is directly proportional to blood glucose concentration and helps determine diabetes control. It provides essential information for adjusting insulin dosage and meal patterns. Increased HbA1c levels raise the risk of diabetic complications such as kidney and eye disease, heart disease, stroke, and nerve damage(19). A study by Jouda et al(20). found that AST and ALT levels were significantly higher in hyperthyroidism compared to hypothyroidism and the control group, with no differences between hypothyroidism and the control group. Rao et al(21).observed significant associations between AST or ALT enzyme activity and fasting blood sugar level, age, body mass index, heart rate, and blood pressure. Diabetic patients with secondary complications had significantly higher mean values of AST and ALT compared to those without complications. Patients with a family history of diabetes also had higher mean AST and ALT values compared to those without diabetes. Elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels result from tissue ischemia and hepatocyte infarction due to increased metabolic activity and oxygen demand by the liver(22). Hyperthyroidism affects multiple body systems, including the liver, leading to dysfunction through mechanisms such as toxicity, hypoxia, free radical damage, autoimmune injury, and congestive hepatopathy. Chronic viral hepatitis, including hepatitis B virus (HBV) and hepatitis C virus (HCV), poses significant public health threats as major causes of cirrhosis and hepatocellular carcinoma, particularly in developing countries. Since the liver is crucial for glucose metabolism, its dysfunction due to chronic hepatitis can complicate diabetes management, especially in patients with chronic hepatitis B infection(23) .

4. Conclusion

In conclusion, TSH regulation is crucial for metabolic processes, with high TSH indicating hypothyroidism, often linked to obesity and diabetes complications. Elevated HbA1c levels in diabetics reflect poor glucose control and increased complications. Liver enzymes GOT and GPT are higher in hyperthyroidism, and diabetes exacerbates liver stress. Hyperthyroidism affects multiple systems, including the liver, causing dysfunction through toxicity and autoimmune injury. Chronic viral hepatitis also threatens liver function and glucose metabolism, highlighting the importance of understanding these interactions for managing thyroid and diabetic patients.

Ethics

Every study was carried out in accordance with the University of Kirkuk's humane treatment guidelines in Kirkuk, Iraq.

Conflict of Interest

The writers affirm that there is no conflict of interest between them.

Reference

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