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Study of concentrations of some vitamins in women with type 2 diabetes and insulin resistance

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Abstract The current study was aimed to estimate the levels of some physiological and biochemical parameters in women with T2DM and insulin resistance. 150 women with type 2 diabetes and insulin resistance were reported to have insulin resistance at Azadi Teaching Hospital and Republican Hospital from September 2023 to January 2024. Laboratory tests were performed in private laboratories in Kirkuk, Iraq. The volunteers in the current study were divided as follows: 50 healthy women without any diseases as a control group. 75 patients with type 2 diabetes as a second group. 75 women with insulin resistance as a third group. The results showed that the fasting plasma glucose was shown significantly higher (P<0.05) in T2DM group and T2DM with IR group compared with non-obese; fasting serum insulin was significantly increased in T2DM group and T2DM with IR group compared with non-obese. HOMA-IR test value was significantly increased (P<0.05) in T2DM group and T2DM with IR group compared with non-obese). Vitamin D3 concentrations showed a significant decrease (P<0.05) in the serum of type 2 diabetic patients and insulin resistance group compared to the control group. On the other hand, vitamin B12 concentrations showed a significant decrease (P<0.05) in the serum of type 2 diabetic patients and insulin resistance group compared to the control group. Folic acid concentration also showed a significant decrease (P<0.05) in the type 2 diabetic group and the insulin resistance group compared to the control group. It is concluded that the type 2 diabetes and insulin resistance were associated with vitamins deficiency in patients.

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Keywords: *T2DM; insulin resistance; Vitamin D3; Vitamin B12.*

1. INTRODUCTION

According to the World Health Organization (WHO), diabetes is a chronic metabolic disease characterized by high blood glucose levels, which over time leads to damage to the heart, blood vessels, eyes, kidneys, and nerves. More than 90% of diabetes cases are T2DM, a condition characterized by insufficient insulin secretion by pancreatic islet beta cells, insulin resistance (IR), and an inadequate compensatory insulin secretory response [1,2]. Insulin resistance is a condition in which insulin has an abnormal biological effect. In particular, it is characterized by a decreased ability of insulin to induce glucose utilization by muscle and adipose tissue and inhibits hepatic glucose production and output [3]. Several epidemiological studies have concluded that obesity is a major risk factor for T2DM, and obese individuals are said to be up to 80 times more likely to develop T2DM [4]. Furthermore, an inverse relationship between plasma fatty acid concentration and insulin sensitivity has been demonstrated in age- and BMI-matched controls [5]. In diabetic patients, an inverse relationship was observed between vitamin D levels and body mass index, impaired glucose homeostasis, and HOMA-IR. Some studies, including animal models and human subjects, have reported a relationship between vitamin D and both IR and T2DM, suggesting a beneficial effect of vitamin D on obesity, IR, and T2DM [6]. There are several ways in which vitamin D deficiency can affect insulin sensitivity, some of which are still not fully understood. According to several studies, vitamin D improves the transcription and quantity of insulin receptors in insulin-responsive tissues by interacting with vitamin D receptors. In addition, vitamin D deficiency can affect the concentration of extracellular calcium and its influx through the insulin-responsive cell, which in turn activates glucose transporters and improves the cell's response to insulin [7]. Furthermore, vitamin D may prevent the effect of inflammatory cytokines on insulin signaling by suppressing the release of inflammatory cytokines and modulating the immune system [8,9]. So, the current study was aimed to estimate the levels of some physiological and biochemical parameters in women with T2DM and insulin resistance.

2. Materials and Methods

Patients

The study included collecting 150 blood samples from patients diagnosed with insulin resistance (women aged 30-40 years). After taking the patient's data, patients with other diseases, including chronic diseases, were excluded. As for the control sample, blood samples were taken from healthy women with no medical history.

The blood collection

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mechanism included obtaining the patient's consent at the beginning, and then a specialized team drew 5 ml of venous blood after sterilizing the patient's arm. The blood was placed in a test tube with a yellow cap, and then the gel tube was separated from it by a centrifuge. After separating the serum from the blood, the serum was distributed into three Eppendorf tubes for the purpose of storing and freezing it for physiological and immunological analyses [10,11].

Study groups

150 women with type 2 diabetes and insulin resistance were reported to have insulin resistance at Azadi Teaching Hospital and Republican Hospital from September 2023 to January 2024. Private laboratories in Kirkuk, Iraq were used for the laboratory testing. The present study's participants were split up as follows:

• 50 healthy women without any diseases as a control group.

75 patients with type 2 diabetes as a second group.

• 75 women with insulin resistance as a third group.

Measurements

- **Glucose:** A spectrophotometer was used to measure blood sugar in accordance with BioLabo, France's manufacturer's instructions.
- **Human insulin:** insulin ELISA Kit (SUNLONG, China) assays insulin concentration in human serum and plasma using Sandwich-ELISA.
- **Homeostasis Model Assessment Insulin Resistance (HOMA-IR):** HOMA-IR determination by following equation (HOMA-IR= Fasting serum insulin +Fasting serum glucose/405).
- **Vitamin D3 (Vit. D3):** Vit. D3 ELISA Kit (SUNLONG, China) assays Vit. D3 concentration in human serum and plasma using Sandwich-ELISA.
- **Vitamin B12 (Vit. B12):** Vit. B12 ELISA Kit (SUNLONG, China) assays Vit. B12 concentration in human serum and plasma using Sandwich-ELISA.
- **Folic acid:** folic acid ELISA Kit (SUNLONG, China) assays folic acid concentration in human serum and plasma using Sandwich-ELISA.

Statistical Analysis

Microsoft Excel XP and SPSS's Minitab statistical tools were used to statistically evaluate the data. The minimum and highest values, together with the mean ± standard deviation, were displayed for the data. An analysis of variance (ANOVA) test was used to statistically examine the data and determine the significance of the variance between groups [12,13].

3. Results and Discussion

Table (1) shows the distribution of samples and their percentages according to age, where the age group 40-36 years was the highest among patients with type 2 diabetes if it reached 84.0%, and the age group >3040-36 years was the highest among patients with insulin resistance which it reached 74.7%.

Table (1): *Distribution of study samples according to age*

Groups / Age (year)	T2DM patients (75)	T2DM and IR patients (75)
30-35	12(16.0%)	19(25.3%)
36-40	63(84.0%)	56(74.7%)
Total	75(100%)	75(100%)

In Table (1), it was found that with advancing age, the chance of developing type 2 diabetes and insulin resistance increases. The role of advancing age in insulin resistance is clear from clinical observation. It is known that the average age of onset of diabetes is in the mid-forties. With advancing age, the prevalence of type 2 diabetes and impaired glucose tolerance also becomes higher [14]. This phenomenon does not occur due to a single disorder, but due to several factors such as increased body fat and decreased physical activity with advancing age [15]. On the other hand, in the study conducted by Ferrannini et al. [16], it was found that insulin resistance decreased slightly with advancing age, using a periodic test for glucose levels. Their study was conducted on 1146 men and women, ranging in age from 18 to 85 years. It was found that insulin action decreased moderately with advancing age at a rate of 0.9 mmol/min/kg every 10 years. However, after adjusting for BMI, this significance disappeared.

Table (2) shows the distribution of samples and their percentages according to body mass index, where the 25-30 (kg/m2) category was the highest among type 2 diabetes patients, reaching 42.7%, while the >30 (kg/m2)

category was the highest among insulin resistance patients, reaching 70.7%.

Table (2): *Distribution of patients according to their BMI*

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Groups / Parameter	T2DM patients (75)	T2DM and IR patients (75)
BMI < 25 (kg/m2)	17(22.6%)	1(1.3%)
BMI 25-30 (kg/m2)	32(42.7%)	21(28.0%)
BMI >30 (kg/m2)	26(34.7%)	53(70.7%)
Total	75(100%)	75(100%)

In the current study, it was observed that the BMI was in (70.7%) of the cases suffering from obesity (BMI 30 and above), which is more than the study conducted in Oman by Al Hayek and his group (2013), where the BMI was (43.8%) of the diabetic patients suffering from obesity, and (39.3%) suffering from overweight, which is more than the current study (28%). Obesity and type 2 diabetes are associated with insulin resistance. When a person is overweight, the body cells become less sensitive to insulin, especially fat cells [17]. The current study is consistent with other studies [18]. Chachan et al. [19], indicated that obesity is the main cause of insulin resistance, which appears early in the disease and is mainly compensated by hyperinsulinemia. On the other hand, a study by Allinson et al. [20] highlighted the relationship between obesity and insulin resistance, suggesting that systemic metabolic abnormalities may lead to inflammation in some patients. This is consistent with our study findings on higher HOMA-IR values in obese patients, indicating a higher level of insulin resistance. A study by Wondmkun [21] discussed the role of obesity in the induction of insulin resistance-associated diabetes. This is consistent with our study findings on the high prevalence of diabetes in obese patients. A study by Kahn et al. [22] also discussed the mechanisms linking obesity to insulin resistance and type 2 diabetes. The study suggested that in obese individuals, adipose tissue releases increased amounts of unesterified fatty acids, glycerol, hormones, pro-inflammatory cytokines, and other factors involved in the development of insulin resistance.

Table (3) shows the distribution of samples and their percentages according to the educational level of the patients, where the group of type 2 diabetes patients who neither read nor write (illiterates) was higher compared to patients with a higher educational level of patients if it reached 38.7%, and insulin resistance patients also showed that those who neither read nor write had a percentage of 26.6%, which is the highest among the remaining levels.

Table (3): *Distribution of study samples according to educational level*

Groups / Parameter	T2DM patients (75)	T2DM and IR patients (75)
BMI < 25 (kg/m2)	17(22.6%)	1(1.3%)
BMI 25-30 (kg/m2)	32(42.7%)	21(28.0%)
BMI >30 (kg/m2)	26(34.7%)	53(70.7%)
Total	75(100%)	75(100%)

In the current study, a strong association was found between T2DM and insulin resistance with educational level, which increased the risk of T2DM the lower the educational level of patients. Abdulkader [23] found that more than 60% of illiterate or primary school educated individuals were resistant to initiating insulin therapy (p=0.036). The strong association between education and health outcomes clearly supports this finding, which would be more evident if literacy mediates the association between education and glycemic control in such a diabetic population with low household income [24].

Fasting plasma glucose (189.13± 12.03; 159.11± 8.45) was shown significantly higher (P<0.05) in T2DM group and T2DM with IR group compared with non-obese (98.36± 6.52; 4.54 ± 0.13); fasting serum insulin was significantly increased in T2DM group and T2DM with IR group (18.67 ± 1.55; 15.84 ± 1.85) compared with non-obese (7.15 ± 0.93). HOMA-IR test value was significantly increased (P<0.05) in T2DM group and T2DM with IR group (0.513 ± 0.061; 0.432 ± 0.075) compared with non-obese (0.256 ± 0.051) shown in table (4).

Table (4): *the levels of glucose profile in groups*

Groups / Parameter	Control (50)	T2DM patients (75)	T2DM and IR patients (75)	P-Value
Glucose (mg/dl)	98.36± 6.52	189.13± 12.03*	159.11± 8.45	0.001
Insulin levels (µ U/ml)	7.15 ± 0.93	18.67 ± 1.55*	15.84 ± 1.85*	0.001
HOMA-IR test	0.256 ± 0.051	0.513 ± 0.061*	0.432 ± 0.075*	0.001

The major conclusion in this study is that there is a large and progressive rise in HOMA-IR based on BMI, In agreement with other studies [25, 26]. The study by Fernstrom et al. provided an explanation for the rise in HOMA-IR values in obese individuals. They found that people with higher HOMA-IR levels were those who ingested more carbohydrates and sugars than is recommended. Furthermore, they demonstrated that a diet high in seafood, fruits, and vegetables was associated with young people's healthy insulin resistance (HOMA-IR) and lower risk of developing diabetes [27].

Vitamin D3 concentrations showed a significant decrease ($P<0.05$) in the serum of type 2 diabetic patients (14.07±1.55) and insulin resistance group (17.81±1.04) compared to the control group (42.94±4.13). On the other hand, vitamin B12 concentrations showed a significant decrease ($P<0.05$) in the serum of type 2 diabetic patients (147.6±13.75) and insulin resistance group (188.9±10.9) compared to the control group (336.4±21.94). Folic acid concentration also showed a significant decrease ($P<0.05$) in the type 2 diabetic group (5.83 ± 1.62) and the insulin resistance group (8.11 ± 1.07) compared to the control group (16.91 ± 0.84), as shown in Table (5).

Table (5): *Concentrations of some vitamins in the study groups*

Groups / Parameter	Control (50)	T2DM patients (75)	T2DM and IR patients (75)	P-Value
Vitamin D3 (ng/ml)	42.94± 4.13	14.07± 1.55*	17.81± 1.04	0.001
Vitamin B12 (ng/ml)	336.4 ± 21.94	147.6 ± 13.75*	188.9 ± 10.9*	0.001
Folic Acid (ng/ml)	16.91 ± 0.84	5.83 ± 1.62*	8.11 ± 1.07*	0.001

The results of the study showed that serum vitamin D3 levels in women with diabetes and insulin resistance were significantly lower than those in non-diabetic women. In agreement with our results, Calvo-Romero and Ramiro-Lozano [28] reported that vitamin D3 deficiency is common in patients with type 2 diabetes. There are inverse associations between vitamin D3, metabolic control, and insulin resistance. Furthermore, in a study by Saif-Elnasr et al. [29], they stated that "low vitamin D3 levels may play an important role in the development of type 2 diabetes and this finding may have therapeutic implications as cautious vitamin D supplementation may improve glycemic control and oxidative stress in type 2 diabetes. The results also showed that diabetic patients with insulin resistance were vitamin D3 deficient and did not show a significant difference, although a decrease in the diabetic group. Vitamin D3 deficiency is a common problem in developing countries and is responsible for the occurrence of many diseases. Vitamin D3 deficiency in these populations can be attributed to malnutrition, nutritional deficiencies, and poor health. Some authors have reported an association between air pollution, industrial lifestyle, and low seafood intake with low serum vitamin D3 levels. Air pollution may play a role in the higher incidence of vitamin D3 deficiency [30]. Among diabetic patients, lower serum vitamin B12 levels were found in females. This is consistent with Yakubu et al. [31], and Krishnan et al. [32], who revealed lower serum vitamin B12 levels in females with type 2 diabetes compared to the control group. The data of this study showed that patients with diabetes and insulin resistance were folate deficient. These results are similar to those of Malaguarnera et al. [33] who reported that plasma and erythrocyte folate levels were decreased in patients with type 2 diabetes. Plasma and erythrocyte folate levels have been widely accepted as direct biochemical markers of folate status. Therefore, we chose plasma folate because it is known to be a direct marker of folate status in the body. Although patients using metformin were excluded, it has been reported to reduce serum folate levels [34].

4. CONCLUSIONS

It is concluded from the current study that age and body mass index are important factors in the development of diabetes and insulin resistance due to their direct effect on insulin secretion and homeostasis in the body. A link was also found between low vitamins and the occurrence of insulin resistance and type

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2 diabetes, as vitamin D in particular is associated with the regulation of fat metabolism and the regulation of sex hormones, which are directly linked to obesity.

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