ORIGINAL ARTICLE

Assessment of biochemical markers associated with inflammation in patients with type 2 diabetes mellitus

Évaluation des marqueurs biochimiques associés à l'inflammation chez les patients atteints de diabète sucré de type 2

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Abstract

Introduction-Objective: Diabetes mellitus type 2 is a chronic and progressive metabolic disorder characterized by hyperglycemia and long-term inflammation. Vitamin D has anti-inflammatory properties that reduce the production of pro-inflammatory cytokines IL-6 and TNF-α. This study aimed to investigate the role of serum vitamin D, parathyroid hormone, and homeostatic model assessment for insulin resistance (HOMA-IR) on individuals with type 2 diabetes mellitus. Additionally, study the relationship between these factors and interleukin-6 (IL-6), tumour necrosis factorα (TNF-α), and C-reactive protein (CRP).

Method: This case–control study included a total of 100 participants within the age range of 45 to 65 years. There were 50 participants diagnosed with type 2 diabetes mellitus (T2DM) and 50 healthy individuals (control).

Results: The levels of serum IL-6 were significantly increased in the T2DM group compared to the control groups ($p < 0.05$). Additionally, the T2DM group were increased levels of HOMA-IR, TNF-α, and CRP compared to the control group ($p < 0.05$). The patient group showed a decreased in 25-OH vitamin D levels ($P > 0.05$). Although the mean serum PTH level increased, this increase was significantly greater in the T2DM group compared to the control group.

Conclusion: The study revealed a significant negative correlation between vitamin D, HBA1C, and HOMA-IR. There is also a strong positive correlation between HOMA-IR and HBA1c, TNF-α, IL-6, and CRP. This suggests that vitamin D and PTH play a role in glycemic control.

Key words: Vitamin D, Insulin Resistance, Inflammatory Cytokines, HOMA-IR

Résumé

Introduction-Objectif: Le diabète sucré de type 2 est un trouble métabolique chronique et progressif caractérisé par une hyperglycémie et une inflammation à long terme. La vitamine D a des propriétés anti-inflammatoires qui réduisent la production de cytokines pro-inflammatoires IL-6 et TNF-α. Notre objectif était d'étudier le rôle de la vitamine D sérique, de l'hormone parathyroïdienne et de l'évaluation des modèles homéostatiques pour la résistance à l'insuline (HOMA-IR) chez les personnes atteintes de diabète sucré de type 2. De plus, étudiez la relation entre ces facteurs et l'interleukine-6 (IL-6), le facteur de nécrose tumoraleα (TNF-α) et la protéine C-réactive (CRP).

Méthodes: Cette étude cas-témoins a porté sur un total de 100 participants âgés de 45 à 65 ans. Il y avait 50 participants diagnostiqués avec un diabète sucré de type 2 (DT2) et 50 personnes en bonne santé (contrôle).

Résultats: Les taux sériques d'IL-6 ont augmenté de manière significative dans le groupe DT2 par rapport aux groupes témoins ($p < 0,05$). De plus, le groupe DT2 a présenté des taux accrus de HOMA-IR, TNF-α et CRP par rapport au groupe témoin ($p < 0,05$). Le groupe de patients a montré une diminution des taux de vitamine D 25-OH ($P > 0,05$). Bien que le taux moyen de PTH sérique ait augmenté, cette augmentation était significativement plus marquée dans le groupe DT2 comparé au groupe témoin.

Conclusion: L'étude a révélé une corrélation négative significative entre la vitamine D, HBA1C et HOMA-IR. Il existe également une forte corrélation positive entre HOMA-IR et HBA1c, TNF-α, IL-6 et CRP. Cela suggère que la vitamine D et la PTH jouent un rôle dans le contrôle de la glycémie.

Mots clés: Vitamine D, Résistance à l'insuline, Cytokines Inflammatoires, HOMA-IR

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Introduction

Diabetes is a chronic condition that affects the metabolism of carbohydrates, lipids, and proteins. This results in elevated blood glucose levels, referred to as hyperglycemia, which can lead to chronic microvascular and macrovascular complications. Insufficient glucose absorption by cells, leading to diabetes, can occur due to either a reduction in insulin synthesis or a decrease in cells' responsiveness to insulin (1). Individuals diagnosed with type 2 diabetes mellitus (T2DM) have systemic inflammation, impaired functioning of pancreatic beta cells, and elevated blood glucose levels due to insufficient production or ineffective utilization of insulin in their bodies (2). Vitamin D has the potential to decelerate the progression of diabetes type 2 and metabolic syndrome. This can occur either by directly stimulating vitamin D receptors or by indirectly regulating calcium homeostasis (3). The relationship between vitamin D and type 2 diabetes encompasses its influence on insulin production, insulin sensitivity, insulin resistance, calcium metabolism, cytokines, and its role in diabetes progression and consequences (4). Vitamin D, or vitamin D3, is critical for maintaining calcium and phosphorus balance during bone formation and aiding in calcium absorption in the intestines (5). Studies have shown that vitamin D3 has a protective effect on maintaining the bulk of β cells and avoiding apoptosis.

Vitamin D functions as an immunosuppressant by inhibiting the synthesis of pro-inflammatory cytokines, such as interleukin-2, interleukin-12, and TNF, through gene transcription reduction. Type-2 diabetes mellitus (T2DM) is linked to a state of moderate inflammation in the body, defined by elevated levels of cytokines such as TNF and IL-6 in the bloodstream. Inflammation plays a role in the development of insulin resistance (6). IL-6 increases the production of C-reactive protein (CRP), which may play a role in the development of diseases that cause disability, such as osteoporosis, arthritis, and congestive heart failure (7). TNF-α is a protein that affects insulin resistance and lipid metabolism. Checking the levels of this protein may help find type 2 diabetes mellitus (T2DM) early on and lessen its harmful effects (8).

Multiple studies have confirmed a link between elevated levels of parathyroid hormone (PTH) and irregular glucose metabolism, as well as the development of diabetes (9). PTH increases blood calcium levels by promoting bone calcium release, renal calcium reabsorption, and gut calcium absorption (10). In addition, reduced calcium and 25-OH-D concentrations trigger PTH activation. This leads to the stomach's absorption of calcium and the production of 1,25-(OH)-D. This leads to a mutual connection between PTH and VD levels (11). Parathyroid hormone (PTH) can affect insulin resistance by blocking insulin signaling and limiting glucose absorption, whereas vitamin D can improve insulin sensitivity by lowering PTH levels. Furthermore, vitamin D has the ability to alleviate the effects of inflammatory cytokines on insulin signaling by modifying the innate immune system and decreasing inflammatory cytokine secretion (12). This study aims to investigate the relationship between vitamin D levels, insulin resistance (HOMA-IR), and inflammation markers (TNF-α and CRP) in patients with type 2 diabetes.

Methods

This case-control study involved 100 participants with an age range (of 45–65 years), divided into two groups, 50 patients with diabetes mellitus type 2 and 50 healthy individuals (control). We collected blood samples from June 1, 2023, until the end of January, 2024. The Diabetic Clinic and Endocrine Centre at Marjan Medical City Hospital in Babylon Province diagnosed all patients in the study. We drew five milliliters of venous blood from the participants, dividing it into two ml for HBA1C and three ml for serum separation in a gel tube. We left the gel tube to coagulate at room temperature for 20 minutes, centrifuged it at 3000 x g for 20 minutes, and stored the serum in the deep freezer (-80 °C) for later analysis, discarding the hemolysis samples. The enzyme-linked immune sorbent assay method determined serum IL-6, CRP, and TNF-α. We analyzed the levels of calcium, phosphorus, vitamin D, PTH, and insulin, measured the HBA1C using an automated analyzer, and apply Equation 1, (13) to analyze the homeostasis model assessment of insulin resistance [HOMAIR, as shown in Equation (1):

HOMA-IR = [Fasting Insulin (U/ml)×Fasting Glucose (mg/dl)] /405

Equation 1

Inclusion and exclusion criteria

The study's cohort consisted of people with type 2 diabetes mellitus and those without. We have specifically excluded patients with chronic disorders such as liver disease, cardiovascular problems, type 1 diabetes mellitus and rheumatoid arthritis.

We performed all statistical analyses using SPSS version 26. Data were expressed as mean ± SD. The student t-test assessed the normality of all variables' distribution, and we applied Pearson correlation to compare the means between the two groups. A p value ≤ 0.05 was considered statistically significant.

Results

Demographic characteristics of T2DM patients and control groups

The average age of the patients and control group was 45–65 years. The analysis showed no statistically significant difference ($p > 0.05$) in age and body mass index between the T2DM and control groups, suggesting that other factors could account for any variations in the results, as illustrated in Table 1.

The levels of FBS, insulin, (HBA1c), (HOMA-IR), (IL-6), (TNF-α), and (CRP) were all significantly higher ($p<0.05$) in the T2DM group compared to the control group. However, as shown in Table 2, there were significant ($p <0.05$) differences in the levels of serum PTH between the T2DM group and the control group. Also, the T2DM

group had lower levels of 25-OH vitamin D than the control group.

Table 1. Demographic data of T2DM and control groups

Variable	Study groups	Mean± SD	p value
Age (years)	T2DM	52.16 ± 7.378	0.41
	Control	53.55 ± 6.53	
BMI (kg/m²)	T2DM	23.52 ± 2.29	0.08
	Control	22.60 ± 2.09	

Table 2. Comparison between the biochemical parameters of T2DM and the control group

Parameters	Controls	T2DM	p value
FBS (mg/dl)	85.50 ± 2.28	175.86 ± 8.04	0.001
HbA1C (%)	4.63 ± 0.56	8.29 ± 0.52	0.001
Insulin (µU/ml)	8.02 ± 0.54	12.85 ± 0·75	0.001
HOMA IR (%)	2.45 ± 0.69	5.11 ± 0.64	0.001
Calcium (mg/dl)	8.53 ± 0.58	8.07 ± 0.50	0.01
Phosphorous(mg/dl)	3.46 ± 0.31	3.92 ± 0.39	0.03
Vitamin D (ng/ml)	33.96 ± 2.40	19.60 ± 2.01	0.001
PTH (pg/ml)	34.05 ± 8.12	48.87 ± 12.99	0.001
TNF α (pg/mL)	4.98 ± 0.89	5.96 ± 0.80	0.001
IL 6 (pg/mL)	4.42 ± 0.86	10.82 ± 3.86	0.001
CRP (mg/L)	10.67 ± 1.53	15.85 ± 2.17	0.001

Spearman correlation of HOMA IR with other parameters in T2DM patients.

The results showed that HOMA IR had a strong significantly ($p < 0.05$) correlated with HbA1C, TNF-α, IL-6, and CRP. However, it had a non-significant ($p > 0.05$) correlation with insulin, calcium, and PTH, which can be seen in Table 3. The statistical analysis shows a significant positive correlation between serum HOMA IR levels and HbA1C, TNF-, IL-6, and CRP, as shown in Figure 1.

Table 3. Spearman correlation between HOMA IR and other parameters

Parameters	Correlation coefficient (r)	p value
HbA1C (%)	0.294	0.038
Insulin (µU/ml)	0.199	0.167
Calcium (mg/dl)	-0.020	0.889
Phosphorous(mg/dl)	0.347	0.014
PTH (pg/ml)	0.035	0.810
TNF - α (pg/mL)	0.269	0.05
IL- 6 (pg/mL)	0.282	0.047
CRP (mg/L)	0.280	0.049

Spearman correlation of Vitamin D with other parameters in T2DM patients

In patients with T2DM, there was a significant positive correlation between vitamin D and HbA1c, HOMA IR, and PTH ($p<0.05$). A non-significant correlation was found with insulin, calcium, TNF-α, IL-6, and CRP ($p > 0.05$), as shown in Table 4. The statistical analysis shows a significant positive correlation between serum vitamin D and HbA1c, HOMA IR, and PTH, as shown in Figure 2.

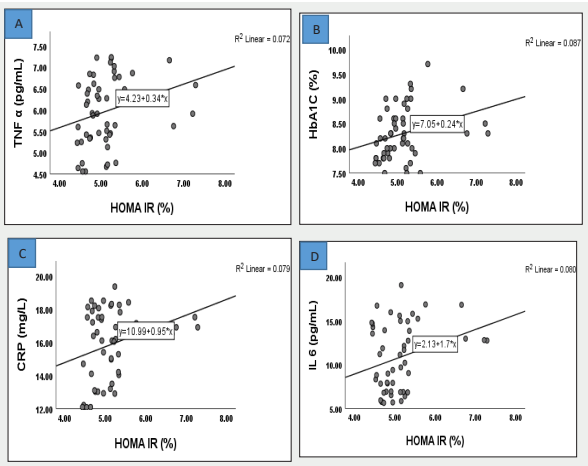

Figure 1. Correlation of serum HOMA IR level versus (A) TNF α (B) HbA1C (C) CRP (D) IL 6

Table 4. Spearman correlation between Vitamin D with other parameters

Parameters	Correlation coefficient (r)	p value
FBS (mg/dl)	-0.044	0.762
HbA1C (%)	- 0.279	0.05
Insulin (µU/ml)	- 0.159	0.270
HOMA IR (%)	- 0.272	0.05
Calcium (mg/dl)	0.178	0.217
Phosphorous(mg/dl)	0.089	0.537
PTH (pg/ml)	- 0.270	0.05
TNF α (pg/mL)	0.221	0.123
IL- 6 (pg/mL)	- 0.191	0.185
CRP (mg/L)	-0.106	0.464

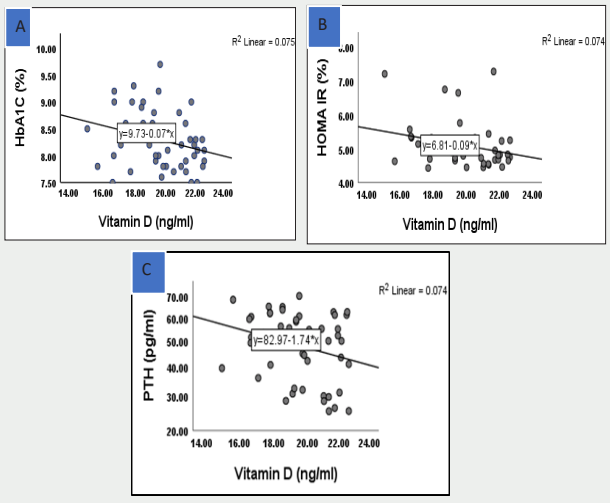

Figure 2. Correlation of serum vitamin D level with (A) HbA1C, (B) HOMA IR, and (C) PTH

Discussion

Type 2 diabetes is a long-term, chronic metabolic disorder that develops gradually characterized by continuous inflammation and high blood sugar levels. The results indicate that diabetic persons with a vitamin D deficiency exhibit poorer control of blood sugar levels, as indicated by their HbA1c levels, and also have increased resistance to insulin, as indicated by their HOMA-IR scores. This

suggests a potential link between vitamin D and glucose homeostasis. We observed a strong association between low levels of vitamin D, elevated mean HbA1c, and fasting plasma glucose levels. Ehsan et al. (14) conducted research that is consistent with these findings. Insulin secretion is dependent on calcium, and insufficient levels of vitamin D impede the ability to release insulin in response to glucose (15).

Vitamin D seems to regulate insulin secretion, irrespective of calcium levels. However, as calcium is essential for insulin release, vitamin D may indirectly exert its effects by controlling the movement of calcium within the cell (16). It is known that when beta cells in the pancreas have central insulin resistance, alpha cells in the pancreas turn on more glucose production. As a result, there is an increase in liver glucose production and a decrease in glucose utilization in peripheral organs (17). The present investigation found that diabetes patients had a higher prevalence of vitamin D insufficiency in comparison to the control groups. In their study, Narayanan S. et al. (18) discovered that persons diagnosed with type 2 diabetes mellitus (T2DM) exhibit reduced levels of vitamin D in comparison to healthy individuals. Vitamin D also has anti-inflammatory effects. Research has shown that vitamin D increases the production of the anti-inflammatory cytokine IL-10 while decreasing the production of the pro-inflammatory cytokines IL6, IL-12, IFN-α, and TNF-α (19).

Vitamin D can induce a decrease in the levels of interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), and other substances. Macrophages and monocytes can regulate these levels. Consequently, this aids in reducing inflammation (20). Our research shows that people with T2DM had much higher amounts of TNF-α and lower amounts of 25(OH)-D in their blood The results are consistent with experimental research that shows vitamin D's ability to decrease the production of TNF-α, a key factor in the development of insulin resistance. This finding supports the idea that the biologically active form of vitamin D can improve insulin release by promoting calcium movement in pancreatic cells. Additionally, a lack of vitamin D may result in widespread inflammation throughout the body (21).

The obtained result was consistent with that reported by Velloso L. et al. (22) stated that high levels of circulating inflammatory biomarkers such as IL-6, TNF, and hs-CRP have been found in people with low vitamin D levels and play an important role in the development of T2DM. Phosat C. et al. conducted research that aligns with the findings of this study (23). A study revealed that CRP predicts the likelihood of developing coronary heart disease, indicating a potential association between abnormal blood sugar levels and coronary heart disease. There were significant associations found between TNF-α and HOMA-β. As a result, the secretion of TNF-α can reduce pancreatic β-cell insulin synthesis. There was a direct relationship between the IL-6 level and the glucose and insulin levels. It is believed to impact the body's glucose homeostasis and metabolic processes, potentially causing indirect effects on adipocytes and pancreatic cells. The outcome demonstrates a strong correlation with the findings of Yin X. et al. (24).

Vitamin D and PTH may both have an impact on glycemic control in individuals with T2DM. Furthermore, increased concentrations of PTH may have a role in the progression of metabolic syndrome (MS), either by directly affecting its components or by adding to insulin resistance (25). Regardless of vitamin D levels research has demonstrated that increased levels of PTH are associated with less insulin sensitivity and a higher prevalence of metabolic syndrome. Our findings on PTH are consistent with previous studies by Mohammed Saeed W. et al. (26-30), which identified an inverse correlation between serum vitamin D, PTH, calcium, and blood glucose levels in T2DM. These results further support the role of both vitamin D and PTH in regulating glycemic control and insulin sensitivity."

Conclusion

The current study indicated that individuals with type 2 diabetes and vitamin D deficiency exhibit higher blood glucose levels, potentially due to decreased beta cell function and its association with insulin resistance (IR). Furthermore, vitamin D levels were inversely correlated with both HbA1c and insulin resistance. The negative relationship between 25(OH) vitamin D3 and glycemic control, coupled with its limited correlation with pro-inflammatory markers, suggests that vitamin D plays a crucial role in the pathophysiology of type 2 diabetes. Additionally, parathyroid hormone (PTH) was found to influence blood glucose regulation. Based on these findings, routine vitamin D monitoring may be beneficial for diabetic patients. Exposure to sunlight, especially in the morning, and the use of vitamin D supplements could be considered as part of an integrated approach to reduce inflammation and support cardiovascular health in diabetes management.

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