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Article

Association between B-Vitamin Levels (B12, B6, Folate) and D with Hyperhomocysteinemia in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Diabetes Mellitus (DM) is a metabolic condition that is increasingly linked to micronutrient deficits. Hyperhomocysteinemia (HHcy), which is caused by vitamin insufficiency, has been identified as an independent risk factor for endothelial dysfunction in Type 2 Diabetes Mellitus (T2DM). The purpose of this study was to look at the relationship between plasma homocysteine levels and vitamin status (B12, B6, folate, and vitamin D) in T2DM patients. This case-control study was carried out at Baqubah Educational Hospital between October 2020 and September 2021. It included 21 healthy controls (mean age 44.90 ± 1.47; 10 men, 11 women) and 70 clinically diagnosed T2DM patients (aged >35 years; mean age 52.94 ± 1.03; 33 men, 37 women). Blood samples were taken following an overnight fast. Enzyme-linked immunosorbent assay (ELISA) kits (SunLong Biotech Co.) were used to assess serum levels of Hcy, vitamin B12, vitamin B6, folate, and vitamin D. The results of the study showed that, in comparison to the healthy group, T2DM patients had significantly higher levels of Hcy ($P < 0.01$). Importantly, compared to controls, T2DM patients had significantly decreased levels of vitamin B12, B6, folate and vitamin D ($P < 0.01$). Based on plasma Hcy levels, patients were divided into two subgroups: T2DM with Hyperhomocysteinemia (T2DM+HHcy, n=48), which is defined as Hcy >15 µmol/L, and T2DM with Normal Hcy (T2DM+NHcy, n=22). In comparison to the T2DM subgroups and the healthy controls, the T2DM+HHcy subgroups had significantly greater Hcy concentrations and lower vitamin levels ($P < 0.01$). In conclusion, T2DM patients are more likely to experience vitamin insufficiency, which is strongly linked to the development of hyperhomocysteinemia. The connection between B-vitamin status and Hcy levels implies that vitamin deficiency could be a key cause to higher levels of Hcy, endothelial dysfunction, and increased cardiovascular risk in these patients.

Keywords Type 2 Diabetes Mellitus; Hyperhomocysteinemia; Vitamin B12; Vitamin B6; Folic Acid; Vitamin Deficiency; Endothelial Dysfunction.

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1. INTRODUCTION

Diabetes is a widespread chronic disease around the world and one of the leading health concerns in most countries. This disease encompasses a range of metabolic disorders (carbohydrate metabolism, protein metabolism, lipid metabolism), the most significant of which is a chronic, long-term elevation in blood glucose levels (hyperglycemia) [1]. A study by Ramarao et al. (2021) indicated that approximately 108 million people had diabetes mellitus in 1980, and this number rose by approximately 382 million to reach about 422 million people with diabetes worldwide in 2016, representing a prevalence rate of 8.5% among adults—double the 4.7% prevalence rate in 1980. Ninety percent of these cases were type 2 diabetes [2]. Diabetes is characterized by a significant or abnormal increase in blood glucose levels caused by a deficiency of the hormone insulin or a decrease in tissue sensitivity to insulin, this can be due to a variety of causes, including psychological or organic factors, or excessive sugar intake, and may result from a dysfunction of insulin receptors caused by genetic or environmental disorders[3]. Diabetes is associated with a range of disorders, including elevated homocysteine (Hcy) levels, which are a risk factor for cardiovascular diseases (coronary, cerebrovascular, and peripheral) and diabetes. The risk associated with high Hcy levels is several times greater than that of other risk factors such as high blood pressure, high cholesterol, and smoking, this is due to a

deficiency in enzymes (cystathionine β-synthase (CBS) or methyl tetrahydrofolate reductase (MTHFR)) or in cofactors (folic acid, vitamin B12) involved in homocysteine metabolism. Consequently, high levels of homocysteine cause atherosclerosis, which occurs directly through the accumulation and oxidation of homocysteine, leading to the formation of free radicals. These free radicals cause damage and tears in the walls of endothelial cells, as well as accelerating the oxidation of low-density lipoproteins (LDL-c), their uptake by macrophages, and the formation of foam cells, leading to the formation of atherosclerotic plaques. This may be accompanied by a tendency toward excessive blood clotting and the formation of clots within the arteries, leading to a reduced blood supply to the heart muscle and causing a heart attack [4,5,6]. Furthermore, most micronutrients—such as vitamins (B6, B9, B12, D) and minerals—also play a role in some way, either as a contributing factor or a consequence of this chronic disease. Consequently, the consequences and complications of diabetes result from an imbalance between the formation of free radicals and their control by natural antioxidants; thus, these micronutrients, such as vitamins, have a very important antioxidant function, and any imbalance in this function leads to the progression of the disease and its complications [7]. Consequently, the current study aimed to investigate the role of hyperhomocysteinemia in relation to vitamin levels in a group of patients with type 2 diabetes.

2. METHOD

The study included 70 T2DM patients (from the educational Baqubah Hospital in Baqubah City, Diyala, Iraq) aged 35 and up, as well as 21 healthy controls. This research was conducted between October 2020 and September 2021. Each participant provided informed consent after being educated about the study and given a special questionnaire to gather data. Blood samples were drawn from the patients and the control group using vein punctures. For ten minutes, five milliliters of blood were left to coagulate at 37 degrees Celsius. After being centrifuged for ten minutes at 3000 Xg, sera were extracted and kept at -20^{C}. SunLong Biotech Co.'s enzyme immunoassay kit (ELISA) was used to assess vitamin B6, B9, B12, D, and homocysteine based on the sandwich principle.

2.1 Criteria for exclusion

Patients with type 1 diabetes, liver disease, endocrine disorders, or a history of neurological or kidney diseases were not included in the study.

2.2 Analysis of Statistics

The Statistical Package for the Social Sciences (SPSS) version 17.0 program was used to analyze the data. The mean ± standard error was used to express the data. The statistical analysis included ANOVA and the t-test to determine group differences. $P \leq 0.05$ was used to define statistical significance.

3. RESULTS AND DISCUSSION

According to table (1), there were 70 T2DM participants (33 men and 37 women) with an average age of 52.94 ± 1.03 years and 21 healthy controls (10 men and 11 women) with an average age of 44.90 ± 1.47 years. Our results demonstrate a substantial difference in biochemical indicator values between healthy individuals and those with type 2 diabetes . When compared to healthy individuals, the homocysteine levels of T2DM patients all significantly elevated ($P \leq 0.01$), as Table 1 illustrates. This finding is consistent with the results of a study by Ye et al. (2021), which showed that elevated homocysteine levels in patients with type 2 diabetes are strongly associated with kidney damage and the development of diabetic nephropathy. This elevation has been identified as a potential serum marker for the early diagnosis of diabetic nephropathy, It also showed that several factors influence Hcy levels in DM, such as 5-methylenetetrahydrofolate reductase, cystathionine-β-synthase, insulin, and the levels of folic acid and vitamin B12 in the body[8]. Another study attributed the causes of hyperhomocysteinemia in patients with type 2 diabetes to impaired methionine metabolism and reduced Hcy clearance, for every 5 µmol/L increase in circulating Hcy concentration, the risk of diabetic nephropathy increases approximately fourfold, along with the risk of oxidative stress-induced damage [9].

The findings of this study are consistent with those of recent studies, which have demonstrated an increasing association between plasma Hcy levels, T2DM, and its vascular complications. These studies have noted differences in plasma Hcy levels between diabetic patients and non-diabetic individuals [10]. This is because plasma Hcy levels are regulated by various factors, including genetic factors, diet, hormone levels, blood pressure, renal dysfunction, and duration of diabetes; any imbalance in these factors leads to elevated Hcy levels and the development of vascular complications in diabetic patients [11].

Table 1. compares biochemical markers between the T2DM patient group and the healthy group.

Parameters	Non-diabetic (healthy) =21 Mean ±SE	T2DM =70 Mean ±SE	P-value
Age (years)	44.90 ± 1.47	52.94 ± 1.03	
Duration of diabetes(years)	-	6.98±0.20	
Homocysteine (nmol/mL)	13.82± 1.68	18.04±0.58	P ≤ 0.01

Table 2 shows the levels of vitamins D, B12, B9, B6 in the group of patients with type 2 diabetes compared to the control group. The results of the current study indicated that vitamin levels in diabetic patients were significantly lower than those in the control group, with a statistically significant decrease in vitamin D3 levels that was significantly associated with lower levels of vitamins B9, B6 in the group of patients with type 2 diabetes compared to the control group, with a p-value of ≤ 0.01.

Table 2. A comparison of vitamin levels between a group of patients with type 2 diabetes and a group of healthy individuals.

Parameters	Non-diabetic (healthy) =21 Mean ±SE	T2DM =70 Mean ±SE	P-value
Vitamin D (ng/mL)	24.12± 3.30	13.20±0.58	< 0.001
Vitamin B9 (ng/mL)	13.57± 1.58	9.12± 0.38	< 0.001
Vitamin B12 (pg/dL)	702.14± 51.83	547.49± 38.04	< 0.001
Vitamin B6 (pmol/L)	1015.18± 30.39	766.45± 28.72	< 0.001

The findings regarding low vitamin D levels in the current study are consistent with the results of a local study conducted by Salih et al. (2021), which reported low vitamin D levels in their participants[12]. Vitamin D plays an important role in reducing chronic metabolic syndromes such as type 2 diabetes and cardiovascular diseases (CVDs) [13], and its deficiency is closely associated with the accelerated development of insulin resistance. Furthermore, low vitamin D levels increase the risk of T2DM and impaired glucose control [14], which is consistent with the results of the current study.

With regard to the low levels of B vitamins observed in the patients in the current study, our findings are consistent with those of recent studies indicating that low levels of folic acid and cobalamin can interfere with DNA synthesis, cause cellular inflammation, and increased lipid synthesis and homocysteine levels in the blood, which leads to the development of cardiovascular and cerebrovascular diseases due to endothelial damage resulting from atherosclerosis, as well as contributing to the development of insulin resistance in diabetic patients [15].

Another study showed that vitamin B12 and certain other B vitamins help lower homocysteine levels, which increase the risk of developing T2DM by promoting oxidative stress, insulin resistance, β-cell dysfunction, systemic inflammation, and endothelial dysfunction. Vitamin B12 deficiency may lead to pernicious anemia, which is frequently associated with T2DM [16].

While it is noted that B6 levels are lower in people with diabetes compared to healthy individuals, numerous studies have reported that it is not surprising that a deficiency in this vitamin may be implicated in many clinically relevant conditions, including autism, schizophrenia, Alzheimer's disease, epilepsy, Down syndrome, cancer, and diabetes, given the large number of reactions in which vitamin B6 is involved [17]. The results of the current study were consistent with those of a study showing significantly lower plasma concentrations of pyridoxine 5′-phosphate (PN), pyridoxine, and pyridoxal (PL) in a group with diabetes compared to the healthy group. The researchers in the study proposed a hypothesis that T2DM may be associated with altered activity of enzymes involved in the interconversion of vitamin B6 derivatives [18].

The group with type 2 diabetes was stratified based on homocysteine levels, according to a study by [19], to demonstrate the role of hyperhomocysteinemia with the studied indicators, as a homocysteine level greater than 15.0 nmol/ml (Hcy) indicates hyperhomocysteinemia (HHcy), and a homocysteine level less than 15.0 nmol/ml (Hcy) indicates normal homocysteine levels (NHcy).

Table 3 shows the levels of vitamins D3, B12, B9, and B6 in the two groups of patients with type 2 diabetes compared to the control group. The results of the current study indicated that vitamin levels in the two groups of diabetic patients were significantly lower than those in the control group, A statistically significant decrease in vitamin D3 levels was observed, which was significantly associated with a statistically significant decrease in vitamin B9 and B6 levels in the two groups of type 2 diabetes patients compared to the control group, with a p-value of ≤0.01.

Table 3. Comparison of vitamin levels according to homocysteine status between two groups of patients with type 2 diabetes and a group of healthy individuals.

Parameters	Diabetic Type 2 +HHcy =48 Mean ±SE	Diabetic Type 2 +NHcy =22 Mean ±SE	Non-diabetic =21 Mean ±SE
Vitamin D (ng/mL)	13.04± 0.70 **a	13.55± 1.04 *b	24.12± 3.30
Vitamin B9 (ng/mL)	9.04± 0.45 **a	9.29± 0.71 *b	13.57± 1.58
Vitamin B12 (pg/dL)	565.27± 53.46 *a	508.71± 32.73 *b	702.14 ± 51.83
Vitamin B6 (pmol/L)	776.43± 33.76 **a	744.68± 55.02** b	1015.18± 30.39

* P-values was considered statistically significant at 0.05; ** P-values was considered statistically significant at 0.01. ns: non-significant; a: significant different between HHcy and control; b:

significant different between NHcy and control; c: significant different between HHcy and NHcy.

The results of the current study are consistent with those of Mutlu et al. (2016), who reported low vitamin D levels in 5,675 participants with diabetes and found that low vitamin D levels were associated with microvascular damage in the retina [20].

With regard to the low levels of B vitamins in both groups of patients with type 2 diabetes, this is consistent with the findings of a study by Keche et al. (2016), who observed a moderate increase in homocysteine levels alongside low levels of folic acid and vitamin B12 in patients with type 2 diabetes [21]. Recently, clear evidence has emerged linking vitamin B9 (folic acid) and vitamin B12 deficiency to elevated homocysteine levels in the blood. At the cellular level, vitamin B12 acts as a cofactor for the enzyme methionine synthase, which catalyzes the conversion of homocysteine to methionine. The overall reaction occurs in the cytosol and converts 5-methyl-tetrahydrofolate (5-methyl-THF) to THF while transferring a methyl group to the amino acid homocysteine to synthesize methionine. THF is then converted into intermediates used in the synthesis of pyrimidine bases for DNA; thus, vitamin B12 deficiency leads to homocysteine accumulation and ultimately affects methionine metabolism to the extent that homocysteine cannot be remethylated back to methionine, causing hyperhomocysteinemia, impaired DNA synthesis, and hematological abnormalities such as macrocytic anemia [22].

Regarding the lower levels of vitamin B6 in both groups of diabetic patients compared to healthy individuals, the results of our study were consistent with several studies indicating that a deficiency in pyridoxal 5′-phosphate may affect diabetes in various ways. One study suggested that PLP deficiency may cause insulin resistance by increasing homocysteine levels due to impaired enzymes such as cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL), which require PLP as a coenzyme [23].

PLP plays a crucial role in the homocysteine pathway as a cofactor for two cystathionine synthase enzymes: in the conversion of homocysteine to cystathionine and cystathionase, and in the synthesis of cysteine from cystathionine. A deficiency of this factor leads to elevated homocysteine (Hcy) levels, which can result in serious health complications, such as atherosclerosis, cancer, diabetes, immune disorders, inflammation, and other health issues [24].

4. CONCLUSION

The results show that hyperhomocysteinemia, which is linked to B vitamin deficiencies (B6, B12, folic acid, and vitamin D), is significantly more common in patients with type 2 diabetes. The inverse relationship between the concentrations of these vitamins and homocysteine levels indicates a critical role of nutritional deficiencies in the aggravation of vascular disease. In this context, elevated homocysteine is an essential indicator of endothelial dysfunction resulting from vitamin deficiency. Therefore, it is strategically important to periodically evaluate nutritional and vitamin indicators in order to identify individuals who are at risk of cardiovascular issues early on, allowing for more effective treatment and prevention.

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REFERENCES

[1] W. N. Hussein and H. I. Mahgoob, "Biochemical study of the insulin degrading enzyme IDE in serum of type 2 diabetes patients," J. Educ. Sci. Stud., vol. 4, no. 14, 2019.

[2] E. K. Mehdi and H. F. Farhood, "Knowledge, attitudes, and practice of complementary and alternative medicine among diabetic patients," Med. J. Babylon, vol. 16, no. 1, p. 13, 2019.

[3] L. M. Ibraheem, B. Z. Ahmmad, A. M. Dhafer, and J. M. Dhafer, "Effect of diabetes mellitus on periodontal health status, salivary flow rate, and salivary pH in patients with chronic periodontitis," J. Baghdad Coll. Dent., vol. 32, no. 2, pp. 12–16, 2020.

[4] J. F. Toole, M. R. Malinow, L. E. Chambless, J. D. Spence, L. C. Pettigrew, V. J. Howard, et al., "Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial," JAMA, vol. 291, no. 5, pp. 565–575, 2004.

[5] S. Korkmaz, A. Yilmaz, G. Yildiz, F. Kiliçli, and S. Içağasioğlu, "Relationship between homocysteine and non-dipper pattern in patients with type 2 diabetes mellitus," Arq. Bras. Endocrinol. Metab., vol. 56, no. 5, pp. 285–290, 2012.

[6] S. Bansal, S. Kapoor, G. P. Singh, and S. Yadav, "Serum homocysteine levels in type 2 diabetes mellitus patients," Int. J. Contemp. Med. Res., vol. 3, no. 11, pp. 3393–3396, 2016.

[7] S. Zatalia and H. Sanusi, "The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus," Acta Med. Indones., vol. 45, no. 2, pp. 141–147, 2013.

[8] B. Ye, X. Zhu, Z. Zeng, X. Ji, and M. Ji, "Clinical significance of serum homocysteine as a biomarker for early diagnosis of diabetic nephropathy in type 2 diabetes mellitus patients," Pteridines, vol. 32, no. 1, pp. 11–16, 2021.

[9] Y. Wang, X. Fan, B. Fan, K. Jiang, H. Zhang, F. Kang, H. Su, D. Gu, S. Li, and S. Lin, "Scutellarin reduce the homocysteine level and alleviate liver injury in type 2 diabetes model," Front. Pharmacol., vol. 11, Art. no. 538407, 2020.

[10] D. E. Platt et al., "Type II diabetes mellitus and hyperhomocysteinemia: a complex interaction," Diabetol. Metab. Syndr., vol. 9, no. 1, pp. 1–7, 2017.

[11] X. Lei, G. Zeng, Y. Zhang, Q. Li, J. Zhang, Z. Bai, and K. Yang, "Association between homocysteine level and the risk of diabetic retinopathy: a systematic review and meta-analysis," Diabetol. Metab. Syndr., vol. 10, no. 1, pp. 1–8, 2018.

[12] Y. A. Salih, M. T. Rasool, I. H. Ahmed, and A. A. Mohammed, "Impact of vitamin D level on glycemic control in diabetes mellitus type 2 in Duhok," Ann. Med. Surg., vol. 64, Art. no. 102208, 2021.

[13] S. Pokhrel, N. Giri, R. Pokhrel, B. D. Pardhe, A. Lamichhane, A. Chaudhary, and M. P. Bhatt, "Vitamin D deficiency and cardiovascular risk in type 2 diabetes population," Open Life Sci., vol. 16, no. 1, pp. 464–474, 2021.

[14] M. Cojic, R. Kocic, A. Klisic, and G. Kocic, "The effects of vitamin D supplementation on metabolic and oxidative stress markers in patients with type 2 diabetes: a 6-month follow up randomized controlled study," Front. Endocrinol., vol. 12, Art. no. 681109, 2021.

[15] T. Ashok et al., "Role of vitamin B12 and folate in metabolic syndrome," Cureus, vol. 13, no. 10, Art. no. e18666, 2021.

[16] J. Kim, C. W. Ahn, S. Fang, H. S. Lee, and J. S. Park, "Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes," Medicine, vol. 98, no. 46, Art. no. e17909, 2019.

[17] R. Contestabile, M. L. di Salvo, V. Bunik, A. Tramonti, and F. Vernì, "The multifaceted role of vitamin B6 in cancer: Drosophila as a model system to investigate DNA damage," Open Biol., vol. 10, no. 3, Art. no. 200034, 2020.

[18] E. Mascolo and F. Vernì, "Vitamin B6 and diabetes: relationship and molecular mechanisms," Int. J. Mol. Sci., vol. 21, no. 10, Art. no. 3669, 2020.

[19] J. Choudhary, B. Fiza, M. Sinha, and R. Mathur, "Serum homocysteine and its association with lipid profile in type II diabetes mellitus," J. Clin. Diagn. Res., vol. 12, no. 5, pp. BC01–BC04, 2018.

[20] U. Mutlu, M. A. Ikram, A. Hofman, P. T. de Jong, A. G. Uitterlinden, C. C. Klaver, and M. K. Ikram, "Vitamin D and retinal microvascular damage: The Rotterdam Study," Medicine, vol. 95, no. 49, Art. no. e5603, 2016.

[21] Y. N. Keche, R. Yegnanarayan, and S. Bhat, "Effect of vitamin B12 supplementation on glycemic control in poorly controlled hyperhomocysteinemic type 2 diabetic patients," Bangladesh J. Pharmacol., vol. 11, no. 1, pp. 50–53, 2016.

[22] W. W. Tesega et al., "Assessment of serum vitamin B12 and folate levels and macrocytosis in patients with type 2 diabetes mellitus on metformin attending Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: a cross-sectional study," Diabetes Metab. Syndr. Obes., vol. 14, pp. 2011–2020, 2021.

[23] Z. Liu, P. Li, Z. H. Zhao, Y. Zhang, Z. M. Ma, and S. X. Wang, "Vitamin B6 prevents endothelial dysfunction, insulin resistance, and hepatic lipid accumulation in ApoE−/− mice fed with high-fat diet," J. Diabetes Res., vol. 2016, Art. no. 4983750, 2016.

[24] K. Stach, W. Stach, and K. Augoff, "Vitamin B6 in health and disease," Nutrients, vol. 13, no. 9, Art. no. 3229, 2021.