

Relationship between vitamin B12, Homocysteine, and Immunological parameters in patients with type 2 diabetes

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Abstract

Aim: To assess the serum vitamin B12, homocysteine, and immunological parameters in patients with T2DM where tacking metformin in Iraq

Methodology: This is an across-section study using case series of 80 patients with T2DM coming to the Baghdad Teaching Hospital, Medical City, between December 2021 and April 2022. The age group for the study was 40 to 70 years. The blood sample was drawn to measure FBS, Insulin, WBC, IL-6, Hcy, folic acid and Serum Vitamin B12. The statistical analysis was conducted using the SPSS statistical package to analyze the data.

Results: According to the results, there is a significant difference among T2DM patients receiving metformin treatment and the control group in terms of serum levels of vitamin B12 and folic acid. These levels also significantly rise in Hcy, FBS, WBC, insulin, and IL-6.

Conclusion: Low levels of vitamin B12 for diabetic patients taking different doses of metformin, as well as significant differences in WBC and IL-6 between patients and the control group.

Keywords: Vitamin B12, Homocysteine, IL-6, WBC, Folic acid, Diabetic Mellitus, Metformin.

INTRODUCTION

Diabetes mellitus type 2 (T2DM) is a chronic, metabolically complex, multifactorial disease that affects numerous organs and has recently spread around the world [1, 2]. T2DM has also been linked to an increased risk of developing Parkinson's and Alzheimer's diseases [3]. In diabetes patients, cardiovascular disease (CVD) is responsible for 75% of all associated deaths, and people with T2D often have a higher chance of having a lower life expectancy. A number of therapies exist to enhance insulin secretion and/or lessen peripheral tissue insulin resistance, which lowers hyperglycemia [4].

First-line treatment for T2DM is the biguanide metformin, which works well both alone and in conjunction with other drugs that lower blood sugar. It normally has few adverse effects, is well-tolerated, and is cost-effective [5]. By the turn of the century, metformin's capacity to safely lower blood sugar levels in diabetic patients had received extensive global documentation[6].

Animal items like red meat, dairy, and eggs are sources of the water-soluble vitamin B12 (cobalamin), the absorption of B12 in the terminal ileum requires the glycoprotein intrinsic factor, which is generated by parietal cells in the stomach, B12 is employed as a cofactor for enzymes that are involved in the creation of DNA, fatty acids, and myelin once it has been absorbed, hematologic and neurologic problems might occur from a B12 shortage as a result[7]. Clinically significant is the evaluation of vitamin B12 insufficiency in T2DM patients, in patients using metformin, it might manifest as peripheral neuropathy and be misdiagnosed for diabetic neuropathy[8]. Though vitamin B12 insufficiency is not common, diabetic individuals have participated in supplementing trials due to the role that folate plays in the etiology of T2DM and the hyperhomocysteinemia that results from it[9].

Methionine metabolism results in the chemical homocysteine (Hcy). It is a sort of necessary amino acid obtained from a regular diet. With the help of cystathionine beta-synthase (CS) and the auxiliary factor vitamin B6, a portion of Hcy binds to serine and produces cystathionine, which is an enzyme-regulated reaction. The majority of Hct is remethylated, though, to produce methionine[10]. Few research have examined the relationship between interleukin-6 (IL-6) and the etiology of type 2 diabetes. On the other hand, a number of studies have found that IL-6 helps T2DM patients' glucose metabolism while also acting as an

anti-inflammatory[11].

The metabolic syndrome, which also includes dyslipidemia, obesity, hypertension, and alterations in hematological markers, includes T2DM. Red blood cells (RBCs), white blood cells (WBCs), platelets (PLT), and the coagulation systems are only a few of the hematological abnormalities that T2DM patients experience[12].

PATIENTS AND METHOD

This study included 120 samples of registered participants 40 – 70 years visiting the Baghdad Teaching Hospital in Medical city between December 2021 to April 2022 .The study was conducted on 80 males and females with T2DM those patients were taking metformin, and divided into two groups, (Group 1) included 40 T2DM patients taking $\leq 1,000$ mg/day, and(Group 2) included 40 T2DM patients taking $\geq 1,500$ mg/day for three years or more, and healthy control group involved 40 samples.

After an overnight fast, T2DM patients and healthy controls (HCs) had 7 mL of blood drawn in the early morning. In a gel tube, 5 mL from fasting blood samples were collected, the serum was separated by centrifugation at 3000rpm for 10 minutes. Also 2 mL was added in a tube containing ethylene diamine tetra acetic acid (EDTA)to measure CBC. s.B12 was measured by the competitive principle of Electro Chemiluminescence immune Assay (Cobas e411, Roche,Mannheim, Germany) and s.Hcy content in the sample can be determined by measuring the complex's fluorescence intensity by Hipro.

RESULTS

Out of 120 sample size, 80 subjects were used metformin dose (≤ 1000 mg/day and ≥ 1500 mg/day) and 40 subjects were healthy control. The mean age of metformin users was(58.35 ± 1.15 VS 54.15 ± 1.18), and control 53.93 ± 1.33 , FBS showed a highly significant increment between healthy subjects and patients with diabetes treated with metformin (79.96 ± 2.18 vs 172.11 ± 6.97 , 164.40 ± 6.71 mg/dl, Vitamin B12 showed a highly significant decrease between healthy subjects and patients (455.08 ± 20.14 vs 284.70 ± 18.16 , 277.62 ± 16.90 pg/ml), folic acid showed significant decrease between healthy subjects and patients (11.19 ± 0.7 vs. 8.27 ± 0.48 , 9.41 ± 0.56 ng/ml, and Insulin showed a highly significant increased between healthy subjects and patients (6.81 ± 0.31 vs. 10.77 ± 0.58 , 14.02 ± 0.82 μ U/ml). IL-6 showed a highly significant increased between healthy subjects and patients (3.67 ± 0.25 vs. 7.93 ± 0.39 , 7.40 ± 0.25 pg/ml), and Hcy showed a highly significant ($P < 0.001$) increment between healthy subjects and patients (10.83 ± 0.38 vs 13.23 ± 0.61 , 16.07 ± 0.63 μ mol/L, while WBC showed significant increment between healthy subjects and patients (6.40 ± 0.20 vs 7.12 ± 0.30 , $7.93 \pm 0.34 \times 10^9$ /L, respectively (table 1).

Vit B12 level was no significant correlation with age, FBS, IL-6 and Insulin. But the presence of a positive correlation between Vit B12 with WBC ($r = 0.366^{**}$, $P = 0.001$). as seen in table 2 and figure 1.

Figure 2 shows the Receiver operator characteristic curve analysis for IL-6. AUC=0.966, S.E=0.016, 95%CL=0.934 to 0.998 and $p \leq 0.0001$. The figure 3 AUC for WBC =0.670 ,S.E=0.0500, 95%CL=0.573 to 0.767 and $p = 0.002$.

Table (1): Clinical characteristics of the study population

Parameters	Patients Groups		Control	P-Value
	Mean \pm SE G 1 No=40	Mean \pm SE G 2 No=40	Mean \pm SE No=40	
Age (years)	58.35 ± 1.15	54.15 ± 1.18	53.93 ± 1.33	0.018
FBS (mg/dl)	172.11 ± 6.97	164.40 ± 6.71	79.96 ± 2.18	0.0001
Vit B12 (pg/ml)	284.70 ± 18.16	277.62 ± 16.90	455.08 ± 20.14	0.0001
Folic acid (ng/ml)	8.27 ± 0.48	9.41 ± 0.56	11.19 ± 0.7	0.003
Insulin (μ U/ml)	10.77 ± 0.58	14.02 ± 0.82	6.81 ± 0.31	0.0001
IL-6 (pg/ml)	7.93 ± 0.39	7.40 ± 0.25	3.67 ± 0.25	0.0001
Hcy (μ mol/L)	13.23 ± 0.61	16.07 ± 0.63	10.83 ± 0.38	0.0001
WBC $\times 10^9$ /L	7.12 ± 0.30	7.93 ± 0.34	6.40 ± 0.20	0.001

*G 1= (Patient ≤ 1000 mg. day), G 2= (Patient ≥ 1500 mg. day).

Table (2): correlation between Vit B12 and variables in T2DM patients group (r-value).

Vitamin B12	R	P-Value
Age (years)	0.093	0.414
FBS (mg\dl)	0.039	0.732
Folic acid (ng\ml)	-0.068	0.551
Insulin (μ U\ml)	0.155	0.171
IL-6 (pg\ml)	-0.005	0.965
Hcy (μ mol\L)	-0.185	0.101
WBC $\times 10^9$ \L	0.366**	0.001

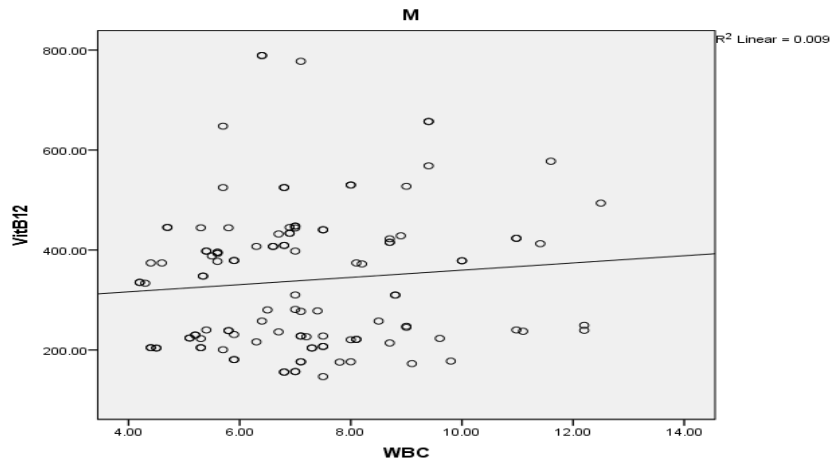


Figure (1): correlation Vit B12 with WBC.

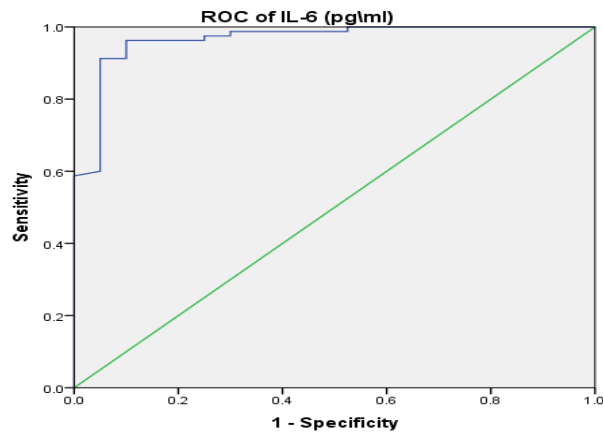


Figure (2): The ROC curve for IL-6.

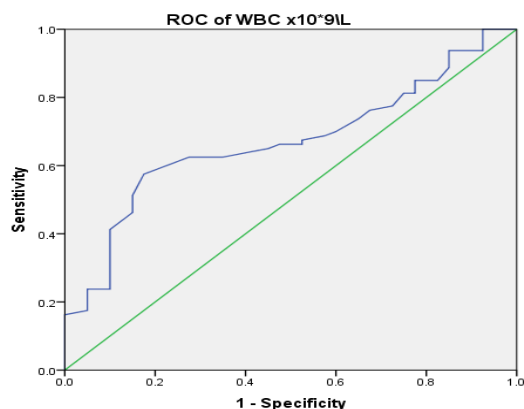


Figure (3): The ROC curve for WBC.

Discussion

It is generally known that people with T2DM who are on metformin have vitamin B12 deficiencies, the medication may prevent calcium-dependent B12 absorption[13].

There is proof that folic acid supplementation may have a favorable impact on markers of oxidative stress and inflammation, however, there is conflicting evidence about the stated effects of folic acid on glycemic management, for instance, people with T2DM who take high doses of metformin may benefit from folic acid supplementation because it lowers plasma concentrations of homocysteine and enhances glycemic control, insulin resistance, and vit B12 status [14].

It has been shown that metformin is linked to a decrease in the level of serum vit B12, Metformin has been demonstrated to increase bacterial overgrowth, modify the bacterial ecology in the intestinal canal, and bind to the Vit B12-intrinsic factor, all of which can result in VitB12 malabsorption (IF), this malabsorption finally causes a drop in serum B12 levels[15].

In line with the findings of earlier research [16,17], metformin therapy reduced fasting plasma glucose concentrations and glucose production by 25–30%. According to several research, metformin mostly inhibits gluconeogenesis[18], which is in line with the findings of our investigation.

It has been demonstrated that metformin added to insulin-based regimens improves glycemic control, restricts changes in body weight, lowers the incidence of hypoglycemia, and reduces insulin demand (sparing effect), allowing a 15–25% decrease in total insulin dosage[19]. As opposed to the Healthy group, the current investigation revealed that metformin administration has distinct effects on cytokines (serum IL-6), these results imply that metformin (1000 mg) used for a year reduces inflammatory reactions in the blood and urine of T2DM individuals [20]. Given that the rate of inflammation in G2 is lower than in G1, this is consistent with our results.

Increased WBC counts are a common indicator of inflammation, and epidemiological studies have found a link between elevated WBC counts and an increased risk of developing diabetes[21]. The WBC count of T2DM patients in the current study was statistically different from the control group, the results of the investigation, which concur with [22], showed a favorable connection between Vit B12 and WBC.

Conclusion:

We discovered that elevated Hcy and vitamin B12 insufficiency are strongly correlated with metformin use at levels equivalent to or higher than 1500 mg/d. Increased WBC counts are a common indicator of inflammation, and epidemiological studies have linked them to a higher risk of diabetes.

REFERENCES

- [1] V. K. P. Venu et al., “Minimizing hyperglycemia-induced vascular endothelial dysfunction by inhibiting endothelial sodium-glucose cotransporter 2 and attenuating oxidative stress: implications for treating individuals with type 2 diabetes,” *Can. J. Diabetes*, vol. 43, no. 7, pp. 510–514, 2019.
- [2] J. Sharifi-Rad et al., “Diet, lifestyle and cardiovascular diseases: linking pathophysiology to cardioprotective effects of natural bioactive compounds,” *Int. J. Environ. Res. Public Health*, vol. 17, no. 7, p. 2326, 2020.

- [3] F. Gentile, P. E. Doneddu, N. Riva, E. Nobile-Orazio, and A. Quattrini, "Diet, microbiota and brain health: unraveling the network intersecting metabolism and neurodegeneration," *Int. J. Mol. Sci.*, vol. 21, no. 20, p. 7471, 2020.
- [4] W. J. English and D. B. Williams, "Metabolic and bariatric surgery: an effective treatment option for obesity and cardiovascular disease," *Prog. Cardiovasc. Dis.*, vol. 61, no. 2, pp. 253–269, 2018.
- [5] C. Baker, C. Retzik-Stahr, V. Singh, R. Plomondon, V. Anderson, and N. Rasouli, "Should metformin remain the first-line therapy for treatment of type 2 diabetes?," *Ther. Adv. Endocrinol. Metab.*, vol. 12, p. 2042018820980225, 2021.
- [6] T. E. LaMoia and G. I. Shulman, "Cellular and molecular mechanisms of metformin action," *Endocr. Rev.*, vol. 42, no. 1, pp. 77–96, 2021.
- [7] A. Ankar and A. Kumar, "Vitamin B12 deficiency," in *StatPearls* [Internet], StatPearls Publishing, 2021.
- [8] M. A. Ahmed, G. Muntingh, and P. Rheeder, "Vitamin B12 deficiency in metformin-treated type-2 diabetes patients, prevalence and association with peripheral neuropathy," *BMC Pharmacol. Toxicol.*, vol. 17, no. 1, pp. 1–10, 2016.
- [9] S. Riaz, "Evaluation and analysis of human folate levels in Pakistani diabetic population," *Int. J. Sci. Eng. Res.*, vol. 5, no. 12, p. 1572, 2014.
- [10] H. Hu et al., "Catalpol inhibits homocysteine-induced oxidation and inflammation via inhibiting Nox4/NF- κ B and GRP78/PERK pathways in human aorta endothelial cells," *Inflammation*, vol. 42, no. 1, pp. 64–80, 2019.
- [11] B. K. Pedersen, "Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease," *Eur. J. Clin. Invest.*, vol. 47, no. 8, pp. 600–611, 2017.
- [12] M. Arkew, T. Yemane, Y. Mengistu, K. Gemechu, and G. Tesfaye, "Hematological parameters of type 2 diabetic adult patients at Debre Berhan Referral Hospital, Northeast Ethiopia: A comparative cross-sectional study," *PLoS One*, vol. 16, no. 6, p. e0253286, 2021.
- [13] A. Khan, I. Shafiq, and M. H. Shah, "Prevalence of vitamin B12 deficiency in patients with type II diabetes mellitus on metformin: a study from Khyber Pakhtunkhwa," *Cureus*, vol. 9, no. 8, 2017.
- [14] P.-H. Lin, W. Aronson, and S. J. Freedland, "Nutrition, dietary interventions and prostate cancer: the latest evidence," *BMC Med.*, vol. 13, no. 1, pp. 1–15, 2015.
- [15] A. Nuzzo et al., "Prevention and treatment of nutritional complications after bariatric surgery," *Lancet Gastroenterol. Hepatol.*, vol. 6, no. 3, pp. 238–251, 2021.
- [16] K. Cusi and R. A. DeFronzo, "Metformin: a review of its metabolic effects," *Diabetes Rev.*, vol. 6, no. 2, pp. 89–131, 1998.
- [17] S. E. Inzucchi et al., "Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus," *N. Engl. J. Med.*, vol. 338, no. 13, pp. 867–873, 1998.
- [18] M. Stumvoll, N. Nurjhan, G. Perriello, G. Dailey, and J. E. Gerich, "Metabolic effects of metformin in non-insulin-dependent diabetes mellitus," *N. Engl. J. Med.*, vol. 333, no. 9, pp. 550–554, 1995.
- [19] S. S. Lund et al., "Combining insulin with metformin or an insulin secretagogue in non-obese patients with type 2 diabetes: 12 month, randomised, double blind trial," *Bmj*, vol. 339, 2009.
- [20] W. Chen, X. Liu, and S. Ye, "Effects of metformin on blood and urine pro-inflammatory mediators in patients with type 2 diabetes," *J. Inflamm.*, vol. 13, no. 1, pp. 1–6, 2016.
- [21] T. Adane, F. Asrie, Z. Getaneh, and S. Getawa, "White blood cells and platelet profiles of diabetic patients at University of Gondar specialized referral hospital: A comparative cross-sectional study," *J. Clin. Lab. Anal.*, vol. 35, no. 6, p. e23808, 2021.
- [22] M. I. Elhabiby, A. M. Tabash, A. M. Yousof, and A. A. H. Mohealdeen, "Correlation Between Metformin and Vitamin B12 level among type II Diabetics in Southern Gaza," *Al-Aqsa Univ. J. (Natural Sci. Ser.)*, vol. 21, pp. 24–43, 2017.