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Article

Association between Serum Vitamin B12 and Folate Levels with Type 2 Diabetes Mellitus On Metformin Therapy

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ABSTRACT

Background: Vitamin B12 is a water-soluble vitamin, and its lack causes the methylation process to be disrupted, further resulting in the formation of abnormal fatty acids, which results in neurological manifestations. The water-soluble vitamin folate, often known as folic acid or vitamin B9, is especially vital during high cell division and growth periods. Objective: The study aims to evaluate serum vitamin B12 and folate levels in type 2 diabetic patients with and without metformin therapy and to correlate their levels with other parameters in type 2 diabetes mellitus (T2DM) patients. Subjects and Methods: A cross-sectional controlled study that included 90 participants, 60 type 2 diabetes mellitus patients with metformin therapy designated as (the metformin group) and 30 type 2 diabetes mellitus without metformin therapy considered as (the control group). The study was conducted from December 2021 to the end of March 2021 at private internal medicine clinics in Tikrit City, Iraq. Blood samples were assessed for serum vitamin B12 and folate using Cl-900i Chemiluminescence Immunoassay Analyzer and glycated hemoglobin HbA1c using AFIAS Analyzer in all metformin and control groups. Result: The study showed that there were significant differences in vitamin B12 of ($p < 0.01$) and folate of ($p < 0.05$) between the metformin group and the control group. Significant negative correlations were found between vitamin B12 and diabetes mellitus (DM) duration and HbA1c. Folate was negatively correlated and highly significant with DM duration and HbA1c ($p < 0.01$). Conclusion: The results showed low vitamin B12 and folate levels in T2DM patients. Vitamin B12 and folate were negatively correlated with DM duration and HbA1c. The results suggest a higher risk of vitamin B12 and folate deficiency with metformin use and emphasize the concern of vitamin B12 and folate deficiency in T2DM patients.

Keywords: Type 2 Diabetes Mellitus (T2DM); Vitamin B12; Folate; DM duration; HbA1c.

INTRODUCTION

Diabetes mellitus (DM) is a major worldwide health problem leading to markedly increased mortality and serious morbidity. It is a significant metabolic illness characterized by chronically elevated blood glucose levels caused by impaired insulin secretion and activity and dangerous consequences [1]. Type 2 diabetes mellitus (T2DM) is a heterogeneous disorder that develops due to reduced response by insulin-sensitive tissues to circulating insulin and defective secretion of insulin by pancreatic beta cells to overcome insulin resistance, giving rise to hyperglycemia [2]. Diabetes is a worldwide disease affecting 90-95% of individuals [3]. Middle East/North Africa has the 2nd-highest T2DM prevalence after Asia [4]. Vitamin B12, also known as cobalamin, a water-soluble vitamin [1], supports hemopoietic, vascular and neuron survival and acts as a coenzyme in single-carbon metabolic pathways. It participates in methionine, pyrimidine, and purine bases synthesis [5]. The water-soluble vitamin folate, often known as folic acid or vitamin B9, is involved in producing deoxyribonucleic acid (DNA) purine bases and converting homocysteine to methionine [6]. The cofactors folate and vitamin B12 regulate homocysteine metabolism [1, 6]. One carbon metabolism requires folate and vitamin B12 to make DNA, Ribonucleic acid (RNA), lipids, amino acids, and neurotransmitters. Folate is reduced and methylated to generate tetrahydrofolate (THF), which takes up carbon to synthesize 5-methyltetrahydrofolate (5-MTHF) in vitamin B12-dependent enzyme [7]. Vitamin B12 transfers the methyl unit from 5-MTHF to homocysteine to form methionine[8], then converted to S-adenosylmethionine (SAM) and homocysteine [9, 10]. The methionine cycle depends on the folate cycle [7]. Functional folate deficiency is caused by vitamin B12 deficiency, which traps free folate as 5-MTHF and accumulates it [9, 11]. Lack of vitamin B12 or folate impairs methionine metabolism, preventing homocysteine remethylation to methionine. Homocysteine turns to cystathionine, then cysteine. Cystathionine-producing enzyme shuts down when cysteine levels rise [7, 9]. Overall, this leads to the accumulation of homocysteine, which is then released into the bloodstream [7]. Lastly, folate and vitamin B12 levels are dropped in conditions of deficiency, respectively, and homocysteine levels rise [1, 12]. Metformin, a medicine of the biguanide class, is one medication that the American Diabetes Association (ADA) suggests as the primary choice for oral therapy in patients with T2DM [13]. Metformin decreases glucose level increments by mechanisms involving delay in glucose absorption from the intestinal lumen, reduction in hepatic gluconeogenesis [14], enhancement in tissue sensitivity towards insulin and augmented glucose uptake in liver, muscle and fatty tissues [1, 15]. Metformin lowers the risk of hypoglycemia by preserving beta cells of the pancreas and restoring insulin secretion [1]. Type 2 diabetes mellitus (T2DM) has been linked to vitamin B12 and folate [16] deficiency. The study aims to evaluate serum vitamin B12 and folate levels in type 2 diabetic patients with and without metformin therapy and to correlate their levels with other parameters in T2DM patients.

MATERIALS AND METHODS

A cross-sectional controlled study included 90 participants: 60 type 2 diabetes mellitus patients with metformin therapy designated as (the metformin group) and 30 type 2 diabetes mellitus without metformin therapy considered as (the control group). The study was conducted from December 2021 to the end of March 2021 at private internal medicine clinics in Tikrit City, Iraq. Investigations included serum vitamin B12, folate, glycated hemoglobin A1c (HbA1c) and DM duration. Serum vitamin B12 and folate levels were measured by Cl-900i Chemiluminescence Immunoassay Analyzer, expressed in units of (pg/ml, ng/ml, respectively) and HbA1c levels by AFIAS Analyzer, expressed in units of percentage (%). Inclusion criteria were diabetic patients aged (20-79 years old), men and women not receiving metformin (for the control group), early diagnosed T2DM, and those

receiving metformin for at least 6 months (for metformin group). Those with type 1 diabetes mellitus, liver and pancreatic disease, documented diagnosed reasons for malabsorption (gastrointestinal disturbances), oral and intramuscular vitamin B12 or folate supplements, consuming alcohol and pregnant women were excluded. The study was approved by the Scientific Committee at Tikrit University - College of Medicine, and the agreement of the attendance to Salah al-Din General for collecting the samples from the patients was approved via the Directorate of Salah al-Din Health. Each patient was educated about the research's purpose, filled out the questionnaire and signed a consent form to participate in the study. Metformin and control groups were subjected to the following biochemical investigations: HbA1c was considered as (normal range = 4.5-6.5 %), and serum vitamin B12 and folate level with a normal value defined as (180-916 pg/ml, 3.20-19.60 ng/ml, respectively). All patients signed an informed consent to participate in the study, and the ethical committee of Tikrit University, College of Medicine approved the study. All data were presented as mean and standard deviation (SD). Statistical analysis was implemented with correlation analysis and t-test. A p-value of less than 0.05 was regarded as significant. IBM SPSS Statistics performed analysis for Windows version 23.0.

RESULTS

The (mean ± SD) age of the metformin group was (51.13 ± 10.76 years), whereas the control group was (49.80 ± 11.29 years). The clinical and biochemical characteristics of diabetic patients are shown in (Table 1). This study showed that there were significant differences in vitamin B12 of ($p < 0.01$) and folate of ($p < 0.05$) between the metformin group and control group, as illustrated in (Table 1).

Parameter	Metformin Group (Mean ± SD)	Control Group (Mean ± SD)	P value
Age (years)	51.13 ± 10.76	49.80 ± 11.29	0.12
DM Duration (years)	4.90 ± 2.67	2.23 ± 1.54	< 0.05
Vitamin B12 (pg/ml)	351.28 ± 110.93	475.55 ± 148.45	< 0.01
Folate (ng/ml)	10.98 ± 2.49	15.76 ± 3.11	< 0.05
HbA1c (%)	8.12 ± 1.14	8.78 ± 1.76	0.25

Table 1. Clinical and biochemical characteristics of studied groups.

Highly significant negative correlations were found between vitamin B12 and DM duration ($r = -0.460$; $p < 0.01$) and HbA1c ($r = -0.412$; $p < 0.01$), as shown in (Figures 1 and 2, respectively). In comparison, folate was negatively correlated highly significantly with DM duration ($r = -0.287$; $p < 0.01$) and HbA1c ($r = -0.281$; $p < 0.01$), as demonstrated in (Figure 3 4, respectively).

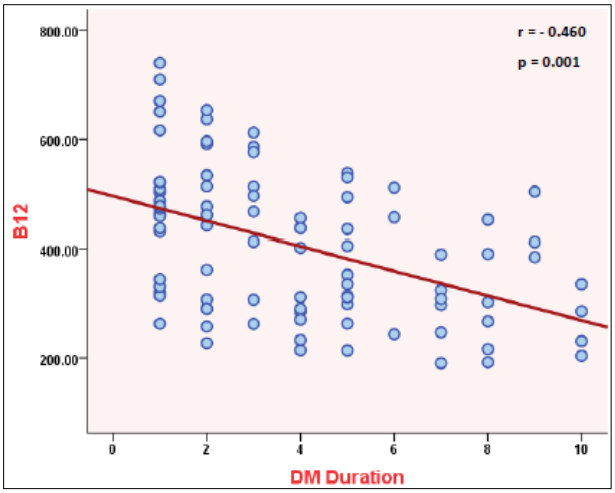

Figure 1. Correlation of vitamin B12 (pg/ml) with (pg/ml) with DM duration (years) in T2DM patients.

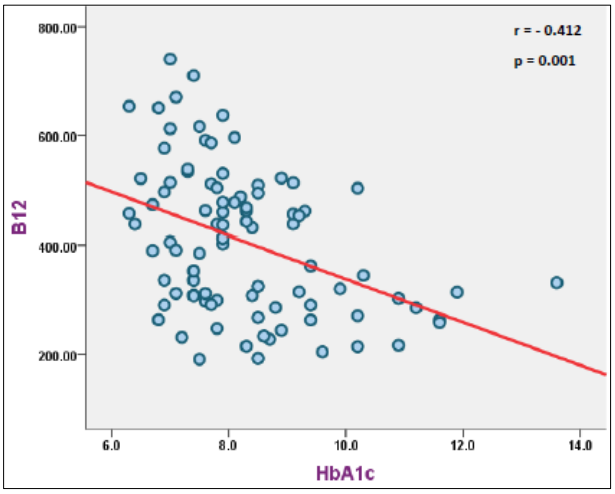

Figure 2. Correlation of vitamin B12 HbA1c (%) in T2DM patients.

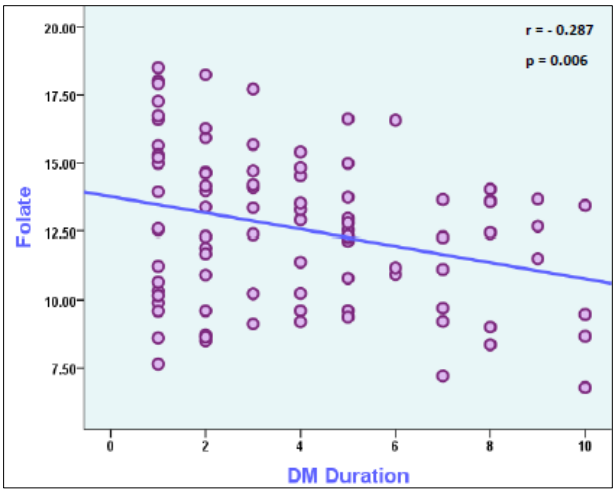

Figure 3. Correlation of folate (ng/ml) with DM duration (years) in T2DM patients.

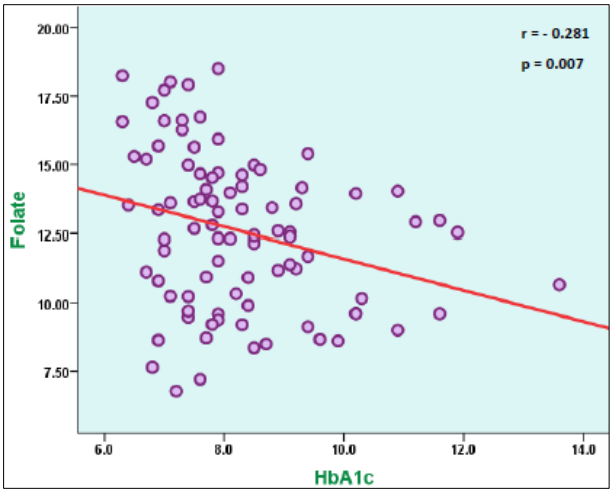

Figure 4. Correlation of folate (ng/ml) with HbA1c (%) in T2DM patients.

DISCUSSION

In the present study, the mean of vitamin B12 was significantly ($p < 0.01$) lower in the metformin group versus the control group, as displayed in (Table 1). Similarly, metformin therapy was reported to be associated with a significant decrease in serum vitamin B12 [12, 17]. Extended use of metformin increases the risk of developing a vitamin B12 deficiency [14]. Metformin suppresses calcium channels in the small intestine, crucial for absorbing the IF-vitamin B12 combination [18]. Lastly, (GIT) atrophy [1], poor absorption and prolonged use of metformin in elderly T2DM patients might explain this significant reduction in vitamin B12 levels. While Sugawara et al. [19] noted that vitamin B12 concentrations did not significantly differ between the control and metformin groups in Japan. The apparent fluctuation may be related to methodological differences, variable locations, age group enrolled, time since diagnosis and duration of metformin treatment, and other nutritional practices. As for folate, the lowest mean was recorded in the metformin group compared to the control group. The difference was significant, as illustrated in (Table 1). Accordingly, it has been noticed that folate levels in T2DM participants were significantly lower in the group receiving metformin [20, 21]. This could be perceived as a simple drop-in serum folate levels as it is influenced by recent dietary intakes [1 6] rather than a real deficit in the tissues. The metformin-associated GIT upset and folate trap hypothesis [9, 11] that results from a true vitamin B12 deficiency, leading to entrapment of folate in 5-MTHF form, is one possible mechanism for folate insufficiency. On the other hand, Karami and Omrani [22] found that folate levels did not change considerably after (3 months) of metformin treatment. This may be due to serum folate level not revealing the true tissue content, which is affected by recent dietary folate [intake 6]. Regarding vitamin B12 correlations, the current study demonstrated a negative correlation between vitamin B12 and DM duration and HbA1c in the metformin group, and the result was highly significant ($p < 0.01$), as shown in (Figure 1-3). The findings are consistent with Almatrafi et al. [23] and Ahmed and Rohman [24], respectively. One cannot rule out vitamin B12 deficiency in long-term DM who seem to have GIT problems related to autonomic neuropathy that cause a decline in intestinal vitamin B12 absorption and subsequent loss in the liver vitamin B12 storage. The function of vitamin B12 in glucose metabolism explains the association between vitamin B12 and HbA1c [25]. Deficiency of vitamin B12 affects glucose, lipid metabolism and oxidative stress [3]. The insulin resistance and anti-glycation products caused by hyperglycemia increase fatty acid metabolism and accumulation in the liver and may disrupt vitamin B12 storage [26]. The fact that the "no metformin group" contained only participants who had never taken metformin excluded the possibility of long-term effects of

metformin. The current study likely shows an intensified inhibition of vitamin B12 absorption, which could cause rapid depletion of the liver store of vitamin B12 in patients taking metformin that may be due to GIT upset and altered intestinal microbiome [3]. However, other studies did not find correlations between vitamin B12 and DM duration [27] and HbA1c [28]. This incompatibility in results might be due to cutoff values, differences in methodologies used in the studies for vitamin B12 assessment and diverse locations, as well as dietary habits and GIT problems, which play a significant role in vitamin B12 levels. Lastly, folate was highly significant and negatively correlated with DM duration and HbA1c in the metformin group ($p < 0.01$). Almost no studies correlated folate level with DM duration and HbA1c deeply; perhaps the obtained negative correlations are because folate and vitamin B12 act synergistically together [6]. Therefore, the reduced vitamin B12 concentration observed in the current study might explain the decreased folate concentration, even though this drop in serum folate levels may be due to decreased recent intakes. Also, finding an inverse relationship between vitamin B12 and hyperglycemia might explain the decline in folate level [26], which may cause functional folate deficiency [9, 10].

CONCLUSION

The results showed low vitamin B12 and folate levels in T2DM patients. Vitamin B12 and folate were negatively correlated with DM duration and HbA1c. The results suggest a higher risk of vitamin B12 folate deficiency with metformin use and emphasize the concern regarding vitamin B12 and folate deficiency in T2DM patients.

Author's Contributions: Student Yasmeen Nasih Tawfeeq; Supervisor1, Nihad Najres Hilal; Supervisor2, Zaidan Jayeed Zaidan. All authors have read and agreed to the published version of the manuscript.

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