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Effect of metformin on vitamin B12 and homocysteine levels among Sudanese with type 2 diabetes mellitus

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Abstract

Metformin is the first-line treatment for type 2 diabetes mellitus (T2DM), and hyperglycemia. When taken in high doses or for an extended period, metformin may decrease vitamin B12 (VitB12) level and may increase homocysteine (Hcy) level. There has been no research conducted in Sudan to determine whether metformin has an impact on VitB12 and Hcy levels in individuals withT2DM. The aim of this research is to investigate the effect of metformin on VitB12 and Hcy levels among Sudanese with T2DM. This cross-sectional study included 98 Sudanese with T2DM who had been taking metformin for at least a year. Data analysis was carried out using SPSS V-26. This study found that 1 patient (1%) had a VitB12 deficiency (< 180 pg/mL), 52 (53.1%) subjects had a normal VitB12 level (180 - 900 pg/mL), and 45 (45.9%) subjects had VitB12 ≥ 900 pg/mL. One patient (2.9%) had an Hcy deficiency (< 3µmol/l) and 34 (97.1%) subjects had a normal Hcy level (3–15 µmol/L). The study showed a, significant negative correlation between VitB12 and vitamin supplements (P = 0.003) and metformin combined with insulin (P = 0.000). Although that belongs to the subgroup that took vitamin supplements, there was a significant positive correlation between VitB12 and metformin alone (P = 0.006) and a significant negative association with metformin combined with insulin (P = 0.000). In the subgroup that did not take vitamin supplements, there was a significant positive correlation between VitB12 and FBS (P = 0.001). There was an insignificant correlation between VitB12 and the metformin dose and use duration. The correlation between VitB12 and Hcy levels was insignificant in the entire group and

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in both subgroups. The correlation between Hcy levels and metformin use duration was significantly negative (P = 0.015), but the correlation between Hcy levels and metformin dose was insignificant. In Sudanese with T2DM and under metformin treatment**,** serum VitB12 level was positively and significantly correlated with DM duration and (FBS), was significantly and negatively correlated with vitamin supplements. On the conversely, serum homocysteine (Hcy) levels significantly decreased with metformin use duration.

Keywords: *T2DM, vitamin B12, homocysteine, metformin, Sudan*

Introduction

Metformin is the initial medication for type 2 diabetes mellitus (T2DM) and is useful both alone and in combination with other antihyperglycemic- drugs.[1] It is recommended by the guidelines of the American Diabetes Association and the European Association for the Study of Diabetes.[2] It assists in lowering blood glucose levels by reducing gluconeogenesis in the liver, decreasing glucose absorption by the small intestine, and increasing the peripheral sensitivity of the insulin hormone.[3] As a side effect of metformin, which is more frequent when taken at high doses or for a long time, 1 in 10 people has low vitamin B12 (VitB12) levels.[4] As this effect is unclear, it has caused conflict among researchers. However, metformin is thought to affect the absorption of VitB12 and offers proof that this is the case in a variety of ways, including that it influences bacterial overgrowth. It may also affect bacterial flora by affecting bacterial motility and/or growth by postponing glucose absorption and trying to prevent the intrinsic factor (IF - VitB12 combination from binding to its receptor in the terminal ileum.[5]

Diabetes mellitus (DM) is a chronic endocrine disorder characterized by hyperglycemia caused by a defect in insulin action, an insufficient amount of insulin synthesis and secretion, or insulin resistance.[6] A total of 700 million people are expected to be affected by 2045, up from an estimated 463 million affected in 2019.[7] Sudan is one of the nations with a prevalence of diabetes of more than 12%. In Arica 3.3% of people are estimated having the disease. According to statistics from the World Health Organization (WHO), DM is expected to have the second-highest mortality rate in Africa.[8] Vitamin (B12) or cobalamin (Cbl), is a water-soluble vitamin. It is mostly obtained from animal sources. Cbl is required for the healthy operation of the central and peripheral nervous systems and is involved in the synthesis of the myelin layer, DNA, fatty acids, and erythrocytes.[9-10] VitB12 deficiency is connected with hematological, neurological, and neuropsychiatric health disorders.[11] Hyperhomocysteinemia and dementia are associated with VitB12 deficiency.[12] The potential reasons for VitB12 deficiency include malnutrition; malabsorption; genetic changes in the way that VitB12 is transported in the blood, cellular uptake, or metabolic route[13]; autoimmune diseases or pernicious anemia [9]; and some medications such as proton pump inhibitors [14] and metformin. [15]

Hcy is an amino acid that has a sulfur group that is formed by the metabolism of methionine. Through remethylation, the methionine synthase enzyme uses Cbl as a coenzyme to change Hcy into methionine in the cytosolic reaction.[16-17] Hyperhomocysteinemia is a biomarker

for predicting tissue Cbl deficiency and is a risk indicator for DM complications, diabetic neuropathy (DNP), and cardiovascular illnesses. [6, 16,18] Hcy accumulation can lead to damage in the cardiovascular system, oxidative stress, neurotoxicity, and malignancy.[16,18-19]
Therefore, the aim of this study was to investigate the effect of metformin on serum VitB12 and Hcy levels and to determine the correlation between serum VitB12 and Hcy levels with the other study variables.

Materials and methods

In this descriptive cross-sectional study, conducted in Khartoum state from November 20, 2022, to February 9, 2023, 98 Sudanese patients with T2DM (males and females aged 30 or more) who had been using metformin for a year or more and had been regularly attending the internal medicine clinic at Jabir Abu EL-Eiz Diabetes Center were included. The samples were subsequently sent right away to Al-Riyadah Medical Laboratory for examination. Patients with bacterial infections, gastrectomy, pernicious anemia, megaloblastic anemia, malnutrition, and malabsorption; or those who were vegetarians were not included in this study. Pregnant women were also not included.

An organized and presented interview-based questionnaire was used to collect the patients' general information (gender, age), medical history, length of time that they had been taking metformin, the daily dose of metformin, other antihyperglycemic medications they had been taking in addition to the metformin, clinical information, symptoms, and complications of DM. If they consumed vitamin supplements, that is another important factor. Each patient's plasma FBS findings and blood HbA1c level were obtained from the center's records, with the exception of those who did not have a test during the previous three months. Fasting samples of venous blood were analyzed in the lab to assess the serum VitB12 and Hcy levels. An iFlash 1800 immunoassay analyzer, based on a competitive chemiluminescence immunoassay was used to assess the serum vitamin B12 levels. A four-point calibration curve, which is instrument-specific, and a master curve made available by the reagent QR code were used to determine the results. VitB12 levels below 180.00 pg/mL were regarded as deficient; those between 180 and 900 pg/ml as normal, and those over 900 pg/mL as above normal. Diuri DR 7000D; based on an enzymatic cycling colorimetric method was used to evaluate serum Hcy levels. Hcy levels were calculated using the NADH oxidation rate as determined by the glutamate dehydrogenase-coupled reaction. Hcy serum concentrations between 3 and 15 mol/L were regarded as normal, whereas concentrations greater than 15 mol/L were regarded as hyperhomocysteinemia.

Data were processed, analyzed, and summarized using SPSS version 26. The independent and dependent variables were shown in tables using a descriptive statistic, and they were expressed by either frequency (N) and percentage (%) or mean ± standard deviation (SD). The correlation between the dependent and independent variables was obtained using Pearson correlation. The significance level was set at $P < 0.05$.

Ethical approval for this study was previously granted by the research ethics review committees of the University of Medical Sciences and Technology, Jabir Abo EL-Eiz Diabetes Center, and the Ministry of Health, Khartoum State, Directorate General of Curative Medicine, Planning and

Training Unit (**ID: 44 (1) on 9/11/2022**) . Informed consent was obtained from all participants prior to sample collection.

Results

In this study, 53 (54.1%) of the participants were females, and 45 (45.9%) of them were males. The mean age of the participants was 55.52 ± 9.79 years. The mean DM duration was 10.4 ± 7.1 years. The mean serum vitamin B12 (VitB12) level was 905.1 ± 422.3 pg/mL. The mean serum homocysteine (Hcy) level was 7.7 ± 1.8 µmol/L. The mean blood HbA1c was 8.7% ± 2.9%. The mean plasma FBS level was 196.4 ± 77.8 mg/dl. The mean period of using metformin was 8 ± 6.2 years (Table 1). The mean serum VitB12 level in females who participated was 991.9 ± 369.4 pg/mL, and the mean serum Hcy level was 7.3 ± 1.9 µmol/L. The mean serum VitB12 level in males who participated was 802.9 ± 460.6 pg/mL, and the mean serum Hcy level was 8.2 ± 1.6 µmol/L (Table 2).

Serum VitB12 deficiency (<180 pg/mL) was found in just one patient (1% of the patients). Total of 52 (53.1%) participants had serum VitB12 levels that were within the normal range (180–900 pg/mL), and 45 (45.9%) subjects had serum VitB12 levels that were higher than the upper limit of the normal range (> 900 pg/mL). Serum Hcy deficiency (< 3 µmol/L) was found in one patient 1(2.9%). A total of 34 (97.1%) participants had serum. Hcy levels that were within the normal range (3 _ 15 µmol/L).

The participants were divided into six groups based on the amount of metformin; 29 (29.6%) participants were using 500 mg once a day, 27 (27.6%) subjects were using 500 mg twice a day, 10 (10.2%) participants were using 500 mg three times a day, 12 (12.2%) participants were using 850 twice a day, and 6 (6.1%) participants were using 850 mg three times a day (Table 3). In addition, the patients who participated were divided into three subgroups based on the type of blood sugar-lowering medication that they used: the number of patients using metformin alone was 23 (23.5%), those using metformin combined with insulin were 47 (48.0%), and lastly, those using metformin combined with sulfonylureas were 28 (28.6%) (Table 4).

In the entire group or in all participants in this study (n = 98), the Pearson correlation showed that serum VitB12 had a positive significant correlation with all of the following study variables: DM duration (P = 0.042), plasma FBS level (P = 0.007), use of metformin alone (P = 0.040), and metformin with a sulfonylureas (P = 0.032). Serum VitB12 level had a strong negative and significant correlation with the following parameters: metformin combined with insulin (P = 0.000), and vitamin supplements (P = 0.003) (Table 5). There was no correlation between serum Hcy level and the other variables in this study in the entire group (Table 6).

Afterward; when the participants in this study were splatted into two subgroups based on who were taking vitamin supplement and who were not: one that were using metformin along with vitamin supplements, and the other that were using metformin without vitamin supplement. The number of participants in the subgroup who did not take vitamin supplements was 33 (33.7%), and 19

(57.6%) of them were females and 14 (42.4%) of them were males. The mean age was 55.3 ± 11 years. The mean DM duration was 9.5± 6.2 years. The mean metformin use duration was 8.9 ± 6.1 years. The mean serum VitB12 level was 730.7 ± 377 pg/mL. The mean serum Hcy level was 7.2 ± 2.3 µmol/L. The mean blood HbA1c was 8.7% ± 2.6%, and the mean plasma FBS level was 191.5 ± 70.1 mg/dL.

In females in the subgroup that did not take vitamin supplements, the mean serum VitB12 level was 812.8 ± 396.6 pg/mL and the mean serum Hcy level was 6.7 ± 2.4 µmol/L. In males in this subgroup, the mean serum VitB12 level was 619.2 ± 329.9 pg/mL, and the mean serum Hcy level was 8.0 ± 2.1µmol/L.

In addition, 25 (75.8%) of the participants in this subgroup had normal serum VitB12 levels, and 8 (24.8%) had levels above the upper limit of the normal range for VitB12. The serum Hcy levels in this subgroup were as follows: only 1 (5.9%) of the subjects had a deficient serum Hcy level, and 16 (94.1%) had a normal serum Hcy level. In the subgroup did not take using vitamin supplements, the Pearson correlation revealed the following outcomes: there was a strong positive and significant correlation between the serum VitB12 level and the plasma FBS level (P = 0.001) (Table 7). In the subgroup did not take vitamin supplements, there was a negative and significant correlation between the serum Hcy level and the metformin use duration (P = 0.015).

In the last subgroup, which included participants with diabetes who were treated with metformin and who were taking vitamin supplements, the findings were as follows: their number was 65 (66.3%), 34 (52.3%) of them were females, and 31 (47.7%) of them were males. The mean age was 55.7 ± 9.2 years. The mean DM duration was 10.9 ± 7.5 years. The mean metformin use duration was 8.9 ± 6.3 years. The mean serum VitB12 level was 993.7 ± 419.1 pg/mL. The mean serum Hcy level was 8.1 ± 1.1 µmol/L. The mean blood HbA1c was 8.7 ± 3.0%. The mean plasma FBS level was 198.8 ± 81.9 mg/dL. In females in this subgroup, the mean serum VitB12 level was 1,092 ± 316.9 pg/mL, and the mean serum Hcy level was 8.0 ± 1.1 µmol/L. In males in this subgroup, the mean serum VitB12 levels was 885.8 ± 491.1 pg/mL, and the serum Hcy was 8.3 ± 1.2 µmol/L.

In this subgroup, 1 patient (1.5%) had a serum VitB12 deficiency, 27 (41.5%) had a normal serum VitB12 level, and 37 (57.0%) had a serum level above the upper limit of the normal range for VitB12. All of the patients in this subgroup had normal serum Hcy levels.

In the subgroup that took vitamin supplements, the Pearson correlation showed the following findings: there was a strong negative and significant correlation between serum VitB12 levels and metformin combined with insulin (P = 0.000), and a strong positive and significant correlation between serum VitB12 levels and the use of metformin treatment alone (P = 0.006) (Table 8).

The serum Hcy level had an insignificant association with all of these study variables. Furthermore, this study showed that the mean levels of VitB12 and Hcy in the entire group as well as the two subgroups: those who were not on vitamin supplements and those who were on, were

compared according to various antihyperglycemic drug categories. The outcomes were as follows: in the entire group, the mean serum VitB12 level of patients who used metformin alone was (747.4 ± 363.6); the mean serum VitB12 of subjects who used metformin combined with insulin was 1,068.2 ± 423.5, and the mean serum VitB12 level of participants who used metformin combined with sulfonylureas was 760.8 ± 372.2.

In addition, the entire group, the mean serum Hcy level of those using metformin alone was 7.8 ± 1.8, the mean serum Hcy level of participants using metformin combined with insulin was 7.4 ± 2.0, and the serum Hcy level of those using metformin combined with sulfonylureas was 7.8 ± 1.7. In the subgroup that did not take vitamin supplements: the mean serum VitB12 level of those using metformin alone was 672.4 ± 458.4, the mean serum VitB12 level of subjects who used metformin combined with insulin was 814.0 ± 370.9, and the mean serum VitB12 level of participants who used metformin combined with sulfonylureas was 668.1 ± 367.0. In addition in the same subgroup, the mean serum Hcy level of those using metformin alone was 7.7 ± 2.9, the mean serum Hcy level of participants using metformin combined with insulin was 7.0 ± 2.9, and the serum Hcy level of those using metformin combined with sulfonylureas was 7.1 ± 1.7. **

In addition in the last subgroup that took vitamin supplements, the mean serum VitB12 level of patients who used metformin alone was 768.2 ± 345.7, the mean serum VitB12 of subjects who used metformin combined with insulin was 1176.1 ± 402.0, and the mean serum VitB12 level of participants who used metformin combined with sulfonylureas was 853.6 ± 366.7. In addition in the subgroup that took vitamin supplements, the mean serum Hcy level of those using metformin alone was 7.9 ± 0.9, the mean serum Hcy level of participants using metformin combined with insulin was 7.8 ± 1.0, and the serum Hcy level of those using metformin combined with sulfonylureas was 8.7 ± 1.3 (Table 9).

Table 1: Descriptive study of the mean age, DM duration, serum VitB12 level, serum Hcy level, blood HbA1c, plasma FBS, metformin use duration in Sudanese patients with T2DM included in the study

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	98	32	80	55.52	9.79
DM duration (years)	98	1	33	10.4	7.12
VitB12 (pg/mL)	98	129	2000	905.1	422.3
Hcy (µmol/L)	35	1.02	10.40	7.7	1.84
HbA1c (%)	72	4.0	15.0	8.7	2.9
FBS (mg/dL)	98	47	420	196.4	77.8

Metformin use duration (years)	98	1	31	8.9	6.2

Abbreviations: Std, Deviation: standard deviation, DM: diabetes mellitus, VitB12; vitamin B12. Hcy: homocysteine, HbA1c; glycated hemoglobin, FBS; fasting blood sugar, T2DM; type 2 diabetes mellitus.

Table 2: Descriptive table of serum VitB12 levels and Hcy levels according to gender

Gender		N	Minimum	Maximum	Mean	Std. Deviation
Females	**VitB12** (pg/mL)	53	357	1,653	991.9	369.4
	Hcy (µmol/L)	22	1.02	9.50	7.3	1.9
Males	**VitB12** (pg/mL)	45	129	2000	802.9	460.6
	Hcy (µmol/L)	13	4.60	10.40	8.2	1.6

Table 3: Frequency table metformin doses among Sudanese patients with T2DM

Dose (mg/day)	**Frequency**	**Percent** (%)
500 once a day	29	29.6
500 twice a day	27	27.6
500 thrice a day	10	10.2
850 once a day	12	12.2
850 twice a day	14	14.3
850 thrice a day	6	6.1
Total	98	100.0

Table 4: Frequency table of antihyperglycemic drugs in Sudanese with T2DM

Antihyperglycemic drugs	**Frequency** (N)	**Percent** (%)
Metformin only	23	23.5
Metformin with insulin	47	48.0
Metformin with sulfonylureas	28	28.6
Vitamin supplements	65	66.3
No vitamins supplement	33	33.7
Total	98	100

Table 5: Correlation table of serum VitB12 levels with the age, DM duration, serum Hcy level, plasma FBS, blood HbA1c, metformin alone treatment, metformin combined with insulin, metformin combined with sulfonylureas, VitB12 supplements, metformin dose /day, and metformin use duration

Parameters	**Pearson correlation value**	**P value** (2 sig)
Age	_0.124	0.225
DM Duration	0.206	0.042*
Hcy	_0.172	0.323
FBS	0.271	0.007

HbA1c	0.226	0.057
Metformin alone treatment	0.208	0.040*
Metformin combined with insulin	_0.373	0.000*
Metformin combined with sulfonylureas	0.217	0.032*
VitB12 supplements	_0.296	0.003*
Metformin dose / day	_0.009	0.926
Metformin use duration	0.112	0.273

Table 6: Correlation table of serum Hcy levels with the age, DM duration, serum VitB12 level, plasma FBS, blood HbA1c, metformin alone treatment, metformin combined with insulin, metformin combined with sulfonylureas, VitB12 supplements, metformin dose/ day, and metformin use duration

Parameters	**Pearson correlation value**	**P value** (2 sig)
Age	_0.058	0.741
DM Duration	_0.096	0.583
VitB12	_0.172	0.323
FBS	0.080	0.649
HbA1c	0.021	0.915
Metformin only treatment	_0.053	0.763
Metformin combined with insulin	0.103	0.557
Metformin combined with sulfonylureas	_0.063	0.718
VitB12 supplements	_0.247	0.152
Metformin dose/ day	_0.110	0.531

Metformin use duration	_0.207	0.234

Table 7: Correlation table of serum VitB12 levels with age, DM duration, serum Hcy, plasma FBS, blood HbA1c, metformin alone treatment, metformin combined with insulin, metformin combined with sulfonylureas, metformin dose/ day, and metformin use duration in the subgroup that did not take vitamin supplements

Parameters	**Pearson correlation value**	**P value** (2 sig)
Age	_0.211	0.239
DM duration	0.103	0.567
Hcy	_0.366	0.148
FBS	0.543	0.001*
HbA1c	0.223	0.332
Metformin alone treatment	0.066	0.714
Metformin with insulin treatment	_0.193	0.282
Metformin with sulfonylureas	0.145	0.422
Metformin dose	0.097	0.590
Metformin use duration	0.070	0.697

Table (8): Correlation table of serum VitB12 levels with the age, DM duration, serum Hcy level, plasma FBS, blood HbA1c, metformin alone treatment, metformin combined with insulin, metformin combined with sulfonylureas, metformin dose /day, and metformin use duration in the subgroup that took vitamin supplements

Parameters	**Pearson correlation value**	**P value** (2 sig)
Age	_0.096	0.445
DM duration	0.219	0.079
Hcy	_0.240	0.338
FBS	0.165	0.188
HbA1c	0.235	0.097
Metformin alone treatment	0.336	0.006*
Metformin combined with insulin	_0.445	0.000*
Metformin combined with sulfonylureas	0.177	0.160
Metformin dose	_0.041	0.747

Metformin use duration	0.138	0.274

Table 9: Comparative table of the mean serum VitB12 and Hcy levels according to the antihyperglycemic drugs in Sudanese with T2DM in the entire group and in both subgroups: those who did not take vitamin supplements and those who did

	VitB12 (180 – 900 pg/ mL)		**Hcy** (3 – 15 µmol/ L)	
Antihyperglycemic drugs	**Mean**	**Std. Deviation**	**Mean**	**Std. Deviation**
In the entire group (n = 98)				
Metformin alone	747.4	363.6	7.8	1.8
Metformin with insulin	1,068.2	423.5	7.4	2.0
Metformin with sulfonylureas	760.8	372.2	7.8	1.7
In the subgroup that did not take vitamin supplements (n = 33)				
Metformin alone	672.4	458.4	7.7	2.9
Metformin with insulin	814.0	370.9	7.0	2.9

Metformin with sulfonylureas	668.1	367.0	7.1	1.7
In the subgroup that took vitamin supplements (n = 65)				
Metformin alone	768.2	345.7	7.9	0.9
Metformin with insulin	1,176.1	402.0	7.8	1.0
Metformin with sulfonylureas	853.6	366.7	8.7	1.3

Discussion

Vitamin B12 (VitB12) is an essential water-soluble micronutrient.[20] It is necessary for the body's growth and development because it participates in a number of metabolic and synthetic processes [21-22], where it works as a coenzyme for certain enzymes and is involved in hematopoietic and neurological functions, as well as DNA synthesis and regulation.[20-22] In the Sudanese population included in this current study, there was a positive significant correlation between serum VitB12 level DM duration (P = 0.042). This finding is in contrast to that reported in Korea by Kim et al (2019), who found that VitB12 significantly decreased with DM duration.[18] In another Sudanese study, Mosab et al. (2020) found an insignificant correlation between serum VitB12 levels and DM duration in Shendi, Sudan.[23] Numerous factors have been linked to VitB12 deficiency in previous studies. Despite its contradictions, the most recent explanation is that numerous studies have demonstrated that metformin, a medication used to treat patients with T2DM, but it led to reduction VitB12 levels when used in high doses or over a long period of time.[24]

This study showed that VitB12 levels increase over time as diabetes progresses. The same result also appeared in the study Mosab *et al* conducted in Shendi, Sudan, but it was statistically insignificant.[23] There are factors that may have contributed to the development of a favorable association between diabetes and VitB12, although this does not necessarily imply that diabetes is the cause of the increase in vitamin B12. For instance, animal sources are affordable and readily available in Sudan in terms of nutrition until recently. In addition, we believe that African DNA may be involved. VitB12 deficiency is common in patients with diabetes, and its decrease may result in a variety of disorders R. The severity of VitB12 deficiency is influenced by the level of VitB12 deficiency as well as other confounding variables, including defects in the synthesis of myelin, DNA, lipids, and RBCs. Hyperhomocysteinemia is another factor of low VitB12 levels. There are numerous more issues and consequences caused by the condition brought by VitB12 deficiency, such as neurological and psychological disorders, anemia, dementia, diabetic peripheral neuropathy, insulin resistance, and finally death.[25-28]

In addition, in the present study, there was a positive significant correlation between serum VitB12 level and plasma FBS in both the entire group and the subgroup that did not take vitamin

supplements (P = 0.007 and 0.001, respectively). This outcome is in contrast to that written by Kim et al. (2019), who found an insignificant relationship between the serum VitB12 level and the plasma FBS.[18] Moreover, the current study showed that serum VitB12 level was negatively correlated with vitamin supplements in the entire group (P = 0.003). This result raises concerns because it is known that taking vitamin supplements improves the body's level of vitamin B12, and the serum VıtB12 levels in the subgroup taking supplements in this study support this theory, when compared to the subgroup that was not using it, this subgroup had higher levels of vitamin B12. This suggests that a number of overlapping factors contributed to the appearance of this outcome. Studying the type and dosage of vitamin supplements is vital to draw a precise conclusion on this issue, as the quantity and type of medication, if not used properly, might have unfavorable effects. The bioavailability of vitamin B12 and absorption and transportation have both been studied. This finding is in contrast to the report of Kim *et al*. who found a positive significant relationship between VitB12 levels and multivitamins.[18]

In the present study, serum VitB12 level was insignificantly correlated with metformin in the subgroup that did not take vitamin supplements. These findings are in agreement with the results of the study by Shahwan *et al.* in Palestine.[11] In addition, in the present study and in the subgroup that did not take vitamin supplements, there was no correlation between serum VitB12 level and metformin combined with sulfonylureas. Shahwan et al. (2020) studied the correlation between VitB12 and sulfonylureas alone, and they showed an insignificant association between them.[11] Furthermore, in this study, the correlation between serum VitB12 level and metformin combined with insulin was insignificant in the subgroup that did not take vitamin supplements. Shahwan et al. (2020) also studied the correlation between VitB12 level and insulin alone and found a significant correlation between VitB12 and the group who took insulin.[11]

Because there are two subgroups in the current study, different results have come out of it. When we compared these results to those of previous studies, we found that; although one subgroup's result may accord with what was showed in earlier studies, the other subgroup's result does not with the some of the study's variables. The subgroup we interested in comparing is those who were not taking any vitamin supplements, because in the previous study, participants were not taking vitamin supplements. For this reason, given the results obtained in the subgroup whose participants were not using vitamin supplements, I found no association between serum VitB12 level and any type of antihyperglycemic drugs. This is consistent with the findings of Mosab *et al*.[23] When comparing the mean for serum VitB12 levels across these antihyperglycemic drugs in the subgroup that did not take vitamin supplements, the mean VitB12 of metformin combined with sulfonylureas was the lowest (668.1 ± 367.0), followed by the mean VitB12 level of metformin alone (672.4 ± 458.4), and the highest one was the mean VitB12 level of metformin with insulin (814.0 ± 370.9). This is in agreement with the results of Mosab *et al*, who reported that the mean VitB12 level of metformin alone is lower than the mean VitB12 level of metformin combined with insulin.[23] In addition, Saber *et al*. conducted a study in Yemen where sulfonylureas were examined independently rather than in combination with metformin, and he reported that VitB12 levels were lower in the sulfonylureas group than in the metformin group.[23]

In general, despite being insignificant, the mean serum VitB12 levels in the entire group as well as the two different subgroups of this study: those who took vitamin supplements and those who did not, are higher in females than males in both of the two subgroups as well as the entire group. The same finding is also reported in Shendi, Sudan, by Mosab *et al.* where the mean VitB12 level in males was lower than that in females [23]. Furthermore, the fact that both studies involved patients with diabetes from Sudan encourages further investigation into the factors that caused the levels of Vit B12 in women to increase in both studies.[23] In the current study, the correlation between serum VitB12 level and metformin dose is insignificant. This result was also showed by Saber *et al*. [29], and Alam *et al* [30], but this finding in contrast to five previous studies reported by Miyan *et al* [7], Al Hamdi *et al*. [31], Kim *et al.* [18], Hashem *et al.* [19] and Sugawara *et al*. [32], who found a correlation between serum VitB12 level and metformin dose in the patients with diabetes.

In addition, in this study, serum Vit B12 levels was insignificantly associated with the metformin use duration. This outcome is in agreement with the results of the study by Saber *et al.* [29], Al Hamdi *et al.* [31], Kim *et al.* [18], Hashem *et al*. [19] and Sugawara *et al.* [32]. In the contrast to this finding are the results of the study by Miyan *et al*. [7] and Alam *et al*. (2021) [30] who showed a correlation between VitB12 level and metformin use duration. This insignificant correlation, may be because the time of this study was very short and the size of the samples was small, and this did not give me much opportunity to study this correlation more broadly. The present study found that there was no correlation between serum VitB12 level and the percentage of HbA1c or the age of the participant. These findings are consistent with several previous studies such as Shahwan *et al.* [11], Kim *et al*. [18], Alam *et al*. [30], Sugawara *et al.* [32], and Al Hamdi *et al.* [31]. In the current study, there was no correlation between serum VitB12 and Hcy levels. This result is in agreement with Sugawara *et al* [32]. In contrast to three previous studies conducted by Miyan *et al.* [7], Kim et al. [18], and Hashem *et al*. [19], they found that the correlation between VitB12 and Hcy was negatively significant.

Homocysteine (Hcy), a nonessential amino acid, plays a vital intermediate role in the metabolism of methionine. Hcy is converted into methionine through the methylation process by the methionine synthase enzyme and coenzyme VitB12. Low VitB12 levels cause an interruption of the methylation mechanism, which leads to accumulating Hcy levels both intracellularly and extracellularly and gravely damages several physiological systems. For instance- but not only- the cardiovascular and nervous systems, which might be a risk factor for diabetic retinopathy and oxidative stress as well as neurodegeneration and age-related macular degeneration.[20, 21, 33] In the current study, particularly in the subgroup that did not take vitamin supplements, the serum Hcy levels significantly decreased with metformin use duration ($P = 0.015$). This finding disagrees the findings of Sugawara *et al.*, who reported that there was an insignificant relationship between Hcy and metformin use duration.[32] In addition, in this current study, there was an insignificant correlation between serum Hcy levels and metformin dose. The same result was also reported in Japan by Sugawara *et al*.[32] In this study, serum Hcy levels were higher in males than in females in all subgroups and the entire group, but not the elevation is insignificantly.

Conclusion

The serum VitB12 levels in Sudanese patients with T2DM who were using metformin as an antihyperglycemic drug significantly increased with both DM duration and plasma FBS levels. By

contrast, serum VitB12 levels significantly decreased with vitamin supplements. Serum VitB12 level is negatively and insignificantly correlated with serum Hcy level. The mean VitB12 level of the combination of metformin and sulfonylureas was the lowest, followed by the mean VitB12 level of metformin alone, and the highest was the mean VitB12 level of the combination of metformin and insulin, which was in the subgroup that did not take vitamin supplements. Serum VitB12 level was insignificantly higher in females than in males. There was no correlation between serum VitB12 levels and metformin dose or use duration. By contrast, the serum Hcy level was a negatively significantly correlated only with metformin use duration, but not with the dose. Serum Hcy was insignificantly higher in males than in the females.

List of abbreviations: Std, Deviation: standard deviation, DM: diabetes mellitus, VitB12; vitamin B12. Hcy: homocysteine, HbA1c; glycated hemoglobin, FBS; fasting blood sugar, T2DM; type 2 diabetes mellitus.

Declarations

Ethics approval and consent to participate: Ethical approval for this study was previously granted by the research ethics review committees of the University of Medical Sciences and Technology, Jabir Abo EL-Eiz Diabetes Center, and the Ministry of Health, Khartoum State, Directorate General of Curative Medicine, Planning and Training Unit (**ID: 44 (1) on 9/11/2022**) . Informed consent was obtained from all participants prior to sample collection. (Attached)

Consent for publication: authors agreed this data to be published (Bulletin of National Research Centre)

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