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The Effect Of Metformin On The Absorption Of Vitamin B12, Folic Acid, Homocysteine And Creatinin; A Cross-Sectional Study In Karbala Province

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ABSTRACT

Recent studies have related to estimate the effect of metformin and other anti-diabetic medication on the level of vitamin B12, folic acid, creatinin and homocysteine in patients with diabetes-mellitus type 2. This cross-sectional study will be accomplished over patients with different parameters including weight, age, body mass index, fasting blood glucose, HbA1c, duration of metformin usage, and the dosage of metformin. The results will be studied under light of control group. Samples size of 300 diabetic patients of type 2, with age ranged between 35-75 years, and a control group consisting of 30 healthy people will be collected from Karbala province/Iraq. As a result, the diabetic patients that taking metformin recorded higher value of homocysteine, and lower value was recorded in term of vitamin B12 and folate in comparison with patients on other antidiabetic drugs or patients on baseline (recently diagnosed). Also, there is not statistically differences in the term of (age, weight, BMI and creatinin) between the groups under studying, meanwhile a statistically differences were observed in term of HbA1c and fasting blood glucose between the investigated groups. Further, the results revealed that the duration and the dose of metformin have considerable impact over vitamin B12 deficiency deterioration.

Objective: Investigating the effect of metformin and other antidiabetic drugs (OAD) on Vit B12, folic acid, fasting blood glucose (FBG), creatinin, glycated hemoglobin (HbA1c) and homocysteine in patients suffering from diabetes mellitus-type 2

Method: The present study was conducted over Karbala province/ Iraq as a cross sectional area of study. The duration of study is specified by the interval between the beginnings of April 2022 to the end of October 2022. The experimental tests were performed in private sector Laboratory.

Result: the diabetic patients that taking metformin recorded higher value for homocysteine, and lower value was recorded for both vitamin B12 and folate in comparison with patients on other antidiabetic drugs or patients on baseline (recently diagnosed). Also, there is not statistically differences in the term of (age, weight, Body Mass Index (BMI) and creatinin) between the groups under studying, meanwhile a statistically differences were observed in term of HbA1c and fasting blood glucose between the investigated groups. Further, the results revealed that the duration and the dose of metformin have considerable impact over vitamin B12 deficiency deterioration.

Conclusion: Patients with DM-type 2 on metformin have high level of Vit. B12 deficiency than patients didn't used metformin or patient on other antidiabetic drugs also Dose and duration play an important role in Vit. B12 deficiency, as the duration and dose increase Vit. B12 deficiency increase.

1. INTRODUCTION

Diabetes-mellitus (DM) is one of the widely spread endocrine diseases and health problems in the growing world today (Green et al. 2005) . According to estimations, in 2017 around 425 million patients suffering from diabetes over worldwide, and it is anticipated by 2045 the observed number of diabetic patients will hitting 693 million, in the same manner, an approximation study revealed that four million of mortality was yearly recorded due to diabetes, that what putting diabetes among the top ten reasons for mortality worldwide (Smokovski 2021). For this reason, extensive studies have been carried out in (last years and currently) in most societies to provide strategies to prevent, relieve, and/or reduce the symptoms and complications of this disease(Allen 2009). The main reasons of DM-type 2 included insulin resistance and/or beta cell activity

loss. It is known that insulin resistance can be enhanced by lifestyle modifying such as regular exercise and loss of weight, and pharmacological treatment to reach to the goal. Today, the 1st line therapy for DM-type 2 is metformin. It is the only medication in the biguanide class of current hypoglycemic drugs. It is characterized by; safe drug with low risk of hypoglycemia, easy access and low cost, and it is associated with decreased cardiovascular risk and death (Powers and DAlessio 2012). Because metformin improves insulin-mediated glucose absorption into skeletal muscle and adipose tissue and decreases hepatic glucose synthesis and release, it lessens insulin resistance. Some investigations have alleged that metformin might interfere with absorption of vitamin-B12 (Vit. B12) and lead to Vit. B12 deficiency (Pratama, Lauren, and Wisnu 2022). In this cross sectional study we Investigate the effect of metformin and other antidiabetic drugs (OAD) on Vit B12, folic acid, fasting blood glucose (FBG), creatinin, Glycated hemoglobin (HbA1c) and homocysteine in patients suffering from diabetes mellitus-type 2 in Karbala province.

2. MATERIAL AND METHOD

Patients

This study was conducted over patients with DM-type 2, and age ranged (35-75) years The selective patients divided into four groups**: first**, diabetic patients on metformin, **second**, diabetic patients that recently diagnosed and without taking any drugs **third**, diabetic patients on another antidiabetic drugs (OAD) like: sulphonylurea, Dipeptidyl peptidase (DPP4), **fourth**, healthy peoples without any diseases.

Blood Sample Collection and Apparatus

After 8 hr. of fasting overnight, 7 mL of venous blood under completely sterilized conditions were acquired from DM-type 2 patients and controls, then, the collected blood samples were divided into 2 groups, first group consist of (2mL) of blood which kept in Ethylene dopamine tetra acetic acid(EDTA-tube) for measurement of HbA1c, and second group consist of (5 mL) of blood which transferred to gel tube to be clot at room temperature by centrifuging to separate it at 3000 (rpm) for ten minutes, in order to obtain a serum that should protected in (-20°C) to be use in biochemical test.

The blood that kept in EDTA-tube are used in the measurement of HbA1c by ARCHITECT c4000 device depending on enzymatic method, while the plasma that we get after centrifugation are used in the measurement of Vit. B12, homocysteine, folic acid, by ARCHITECT i1000 device using Chemiluminescent Microparticle Immuno Assay(CMIA) method. Creatinin is measured by ARCHITECT c4000 device using Alkaline Picrate.

Statistical Analysis

All data are transferred to the computer for statistical analysis ,SPSS version (20; IBM) statistical analysis program was used. Independent t-test utilized to make a comparison between the two groups of each dose and duration of metformin on Vit. B12. Kruskal Wallis test was utilized to make comparison in parameters between the studied groups)more than two group). Pearson's correlation coefficient was utilized to detect a correlation between Vit. B12 and other studied parameters. Data were represented as mean ±standard deviation and P value below than 0.05 was considered as significant depending on statistical approach.

3. RESULTS

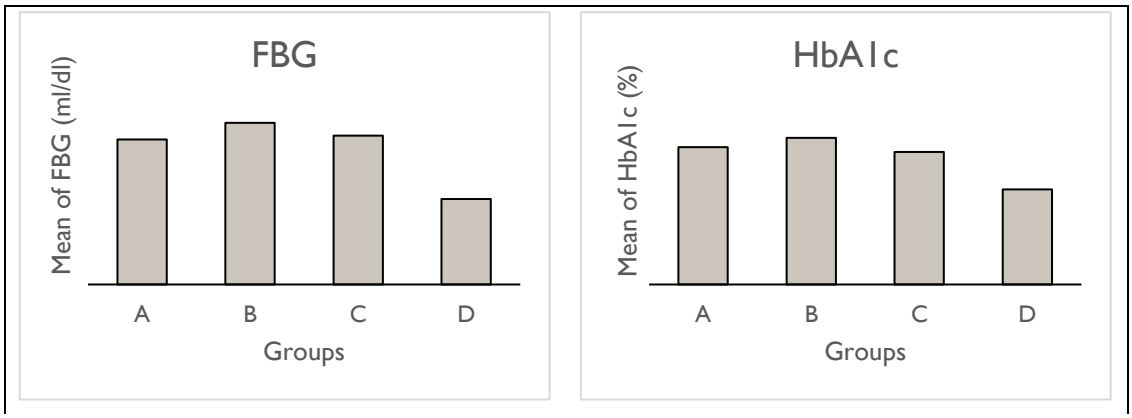
The patient enrolled in this research sorted into 4 groups, the first group (group A)consist of 100 patients with DM-type 2 and taking metformin, the second group (group B) consist of 100 patients with DM-type 2 at baseline and without taking metformin as a medication, the third group (group C) consist of 100 patients with DM-type 2 and taking OAD ,the fourth group (group D) is a control group, that gathered from 30 healthy people without any disease.

the diabetic patients that taking metformin recorded higher value for homocysteine, and lower value was recorded for both vitamin B12 and folate in comparison with patients on other antidiabetic drugs or patients without metformin usage. Also, there is not statistically differences in the term of (age, weight, BMI and creatinin) between the groups under studying, meanwhile a statistically differences were observed in term of HbA1c and fasting blood glucose between the investigated groups. Results are shown in Table (1).

Table 1 :mean of some parameters for the studying groups

Parameter	Diabetic patient Use metformin N=100	Diabetic patient Not use metformin N=100	Diabetic patient Use (OAD) N=100	Control Group N=30	P value
Age (Years)	59.93(±9.77)	58.32(±10.59)	59.20(±10.38)	58.00 (±10.72)	0.67 [NS]

Weight (kg)	86.16(±13.94)	88.33(±13.15)	87.35(±17.81)	85(±11.90)	0.63 [NS]
BMI kg/m²	28.28 (±4.72)	29.22 (±4.25)	28.52(±4.49)	27.85 (±3.98)	0.34 [NS]
Fasting blood glucose mg/dL	165.17(±56.78)	184.14(±56.33)	169.4(±53.43)	97.50(±9.34)	<0.001 [S]
Glycated hemoglobin HBA1C (%)	7.82±0.63	8.35±0.70	7.55±0.46	5.41±0.59	<0.001 [S]
Creatinin (mg/dL)	0.94±0.2	0.91±0.20	0.93±0.20	0.90±0.19	0.74 [NS]
Homocysteine (µmol/L)	16.11±3.47	11.13±2.40	12.36±2.98	11.52±2.70	<0.001 [S]
Folic acid (ng/mL)	12.07±1.02	14.51±1.85	13.7±2.56	14.21±3.17	<0.001 [S]
Vit B12 (pg/mL)	413.07±244.20	527.53±218.99	522.45±213.43	530.85±146.45	<0.001 [S]



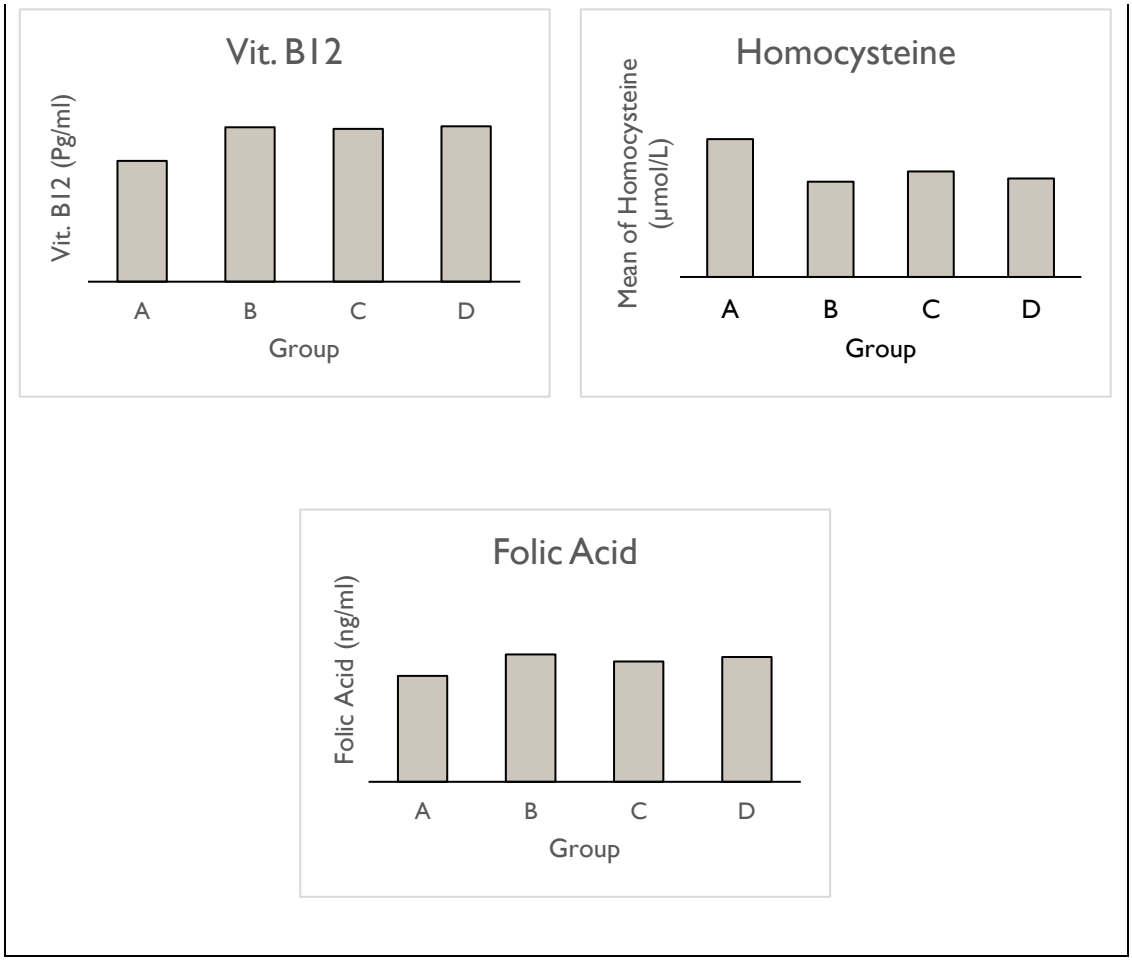


Figure 1: the mean of FBG,HbA1C,Vit B12,Homocysteine,and Folic Acid for the studying groups

Effect of The Duration of Metformin Use on the Vit. B12

The patients using metformin were divided into two groups: 65 using metformin for 5 years or longer, and 35 were using metformin for less than 5 years, Vit. B12 was observed in the two groups according to the duration of metformin usage. The mean of Vit. B12 level of those with metformin use for 5 years or more was lower than those with metformin use for less than 5 years. A significant statistical difference was notice between the two groups as illustrated below.

Table 2: Association between duration of Metformin use and the Vit. B12

duration	**5 year or more than 5 years**	**Less than 5 years**	**P value**
Vitamin b12 pg/mL	370.76±234.4	491.69±245.7	<0.001 [S]

Effect of the dose of metformin on Vit. B12

The patients were divided into two groups depending on the dose of metformin: 56 patients taking metformin >1000 mg/day, and 44 take metformin dose ≤ 1000 mg/day. Vit. B12 was observed in each of the two groups. The mean of Vit. B12 level for group with metformin use <1000 was higher than those with metformin dose >1000. A significant statistical difference was detected as illustrated below

Table 3: Association between the dose of metformin and Vit. B12

dose	more than 1000mg /day	1000 or Less than 1000mg /day	P value
Vitamin b12(pg/ml)	372.91±243.5 pg/ml	464.2± 238 pg/ml	<0.001 [S]

Correlation between Vit. B12 and other parameters

The below Table summarized the correlation between Vit. B12 and age, weight, BMI, creatinin, folic acid, homocystine, HbA1c, FBG, dose and duration of metformin use.

Table 4: Correlation between Vit. B12 and other parameters

Parameters	Correlation (r)	P-value	Significancy
Vit. B12-Age	-0.30	0.002	S
Vit. B12-Weight	-.034	0.73	NS
Vit. B12-BMI	0.040	0.69	NS
Vit. B12-Creatinin	0.066	0.51	NS
Vit. B12-Folic Acid	.076	0.45	NS
Vit. B12-Homocystin	-0.64	<0.001	S
Vit. B12-HbA1c	-0.54	<0.001	S

4. DISCUSSION

Disorders of the glucose metabolism are becoming more commonplace every day. The WHO claims that this rise has reached endemic proportions, particularly in developed and emerging nations. We examine the impact of metformin on the level of vitamin B12, folic acid, creatinine, homocysteine, fasting blood sugar, and HbA1c in patients with type 2 diabetes in the cross section of current study (Association 2010).

The results showed that the age doing a considerable role in Vit.B12 deficiency. Consequently, there is a negative correlation between age and Vit. B12, so when the age increased the level of Vit. B12 decrease (r = -0.30, P = 0.002). The main cause for this correlation is the elderly have low amount of pepsin and intrinsic factor (IF) that needed for Vit. B12 absorption (Quadros 2010) . The result of current study coming compatible with the study of (Loikas et al. 2007) which conducted on 1048 people between 65 and 100 years, they confirmed that Vit. B12 deficiency increased, as age increases. On the other hand, there is no difference in term of weight, BMI, and creatinin between the studied group (p>0.05,(also there is no correlation between Vit. B12 and weight, BMI, and creatinin (r = -0.034, p = 0.73), (r = 0.040, p = 0.69) (r = 0.066, p = 0.51), respectively, these results supported by previous studies (Alsomali et al. 2022; González-González et al. 2022). Recently many hypotheses suggested that the impact of metformin on the Vit. B12 deficiency is attributed to the metformin overlapping with the absorption of Vit. B12-IF complex from the ileal cell in the small intestine, that is calcium-dependent pathway (Pratama et al. 2022).

In this cross-sectional study, we found a significant decrease in the level of Vit. B12 (p<0.001) in 21% patient taking metformin in comparison with patients haven't on metformin or OAD (6%,7%) respectively, this result agreed with the result of (Alharbi et al. 2018; Niafar et al. 2013; Wulffele et al. 2003; Zalaket, Wehbe, and Abou Jaoude 2018). The most consistent risk factor identified in earlier research is the dose and duration of metformin usage, which is connected to the likelihood of developing Vit. B12 deficiency in metformin users. (Ko et al. 2014). In this cross-sectional study, patients on metformin dose more than 1000 (mg/day) have Vit. B12 value lower than patients on metformin dose 1000>by 91.29 (pg/mL), also patients use metformin more than 5 years have lower value of Vit. B12 than patients used ≤5 year by 120.93 (pg/mL). So, there is a significant correlation between Vit. B12 and the dose and duration of metformin (r = -0.53, P<0.001) , (r = -0.47, P<0.001), respectively. Our result is consistent with the study of (Wale Tesega et al. 2021). In another study, serum Vit.

B12 levels were not associated with metformin dose but were associated with duration of treatment only (Ting et al. 2006). Also, (Kim et al. 2019) in their study found that Vit. B12 deficiency occur when the metformin dose increases more than 1 mg per day, also they found there is no effect of duration on metformin deficiency.

The value of homocysteine increases in patients take metformin more than patients not on metformin or using OAD, this increase was found significant in our study P <0.001, also there is a strong negative correlation between Vit. B12 and homocysteine (r = -0.64, P <0.001), this result consistent with (Abbas, Thaker, and Ali 2022) results who concluded in his study diabetic patient on metformin group have lower value of Vit. B12 and folate and higher value of homocysteine than control group, this result reinforced by the result of (Sato et al. 2013) who recorded a significant decrease in Vit. B12 for patients using metformin and this led to increase homocysteine in which the hyperhomocysteinemia is the main cause of retinopathy. In our study the mean value of FBG and HbA1c increased in the first three groups for patients with DM-type 2, meanwhile this value retains in normal range for the control group and there is a significant negative correlation between Vit. B12 and HbA1c, and between Vit B12 with FBG (r = -0.54, P<0.001), (r = -0.46, P<0.001), respectively. these results confirmed by the claims of (Gram-Hansen et al. 1990).

Regarding to Folic acid, the results showed decreased significantly in patients on metformin more than other group (P<0.001), but there is no significant correlation between Vit. B12 and folic acid (r =0.076, P=0.45), this results consistent with result of (Wale Tesega et al. 2021).

5. CONCLUSION

1. Patients with DM-type 2 on metformin have high level of Vit. B12 deficiency than Patients didn't used metformin or patient on OAD.
2. The level of homocysteine increased in patients on metformin more than other groups, so homocysteine measurement used as indicator for Vit. B12 deficiency.
3. There is a significant decrease in serum folate in patients on metformin more than Patients didn't on metformin or using OAD.
4. There is no significant effect of metformin on BMI, weight and creatinin in comparison with the other groups.
5. There is a significant difference in HbA1c for the diabetic patients when compared with control people.
6. Dose and duration play an important role in Vit. B12 deficiency, as the duration and dose increase Vit. B12 deficiency increase.
7. There is a negative correlation between Vit. B12 and age.
8. There is a negative correlation between Vit. B12 and homocysteine.
9. There is a negative correlation between Vit. B12 and dose and duration of metformin.
10. There is a negative correlation between Vit. B12 and HbA1c and FBG

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