Journal of Pharmaceutical Research International

31(6): 1-9, 2019; Article no.JPRI.53717
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

An Investigation on Vitamin B12 Serum Levels among Type 2 Diabetic Patients Treated with Metformin Compared to Normal Levels

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Authors' contributions

This work was carried out in collaboration among all authors. All authors red and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2019/v31i630372
Editor(s):
(1) Rafik Karaman, Professor, Bioorganic Chemistry College of Pharmacy, Al-Quds University, Jerusalem, Palestine.
Reviewers:
(1) Denise Bueno, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil.
(2) Mabrouka El Oudi, Hôpital Militaire de Tunis, Tunisie.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53717

Original Research Article

Received 25 October 2019
Accepted 30 December 2019
Published 06 January 2020

ABSTRACT

Background: Given the increasing prevalence of type 2 diabetes in the community and the use of metformin as one of its most common therapies as well as presence of some evidences on a link between the use of this medicine and vitamin B12 levels, we decided to measure and compare serum level of vitamin B12 among the diabetic patients under treatment by metformin and compare with its natural content. Decreased Vitamin B12 levels leads to some clinical problems including anemia, gastrointestinal disorders (diarrhea, constipation, decreased appetite, etc.,), neurological disorders (tingle, numbness, muscle weakness, etc.,) and mental disorders (memory impairment,

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depression, behavioral problems, etc.,). Therefore, we would determine if the duration of metformin use and dosage may be effective on serum levels of vitamin B12 among metformin-treated diabetic patients.

Methods: Serum vitamin B12 level in metformin-treated type 2 diabetic patients referred to Rasoul-e-Akram Hospital and Specialist Clinic of Taban in Tehran in 2016 (306 subjects selected by convenience sampling) was determined and then compared to its normal level.

Results: Of the 306 participants in the study, 120 ones were females (39.2%) and 186 ones were males (60.8%). Also, 150 ones (49%) were healthy and 156 ones (51%) were suffering from diabetes Miletus. There was a significant difference between the type 2 diabetes mellitus and healthy individuals with quantitative variables such as age, height, weight, BMI, serum cholesterol (Chol) level, LDL serum level, HDL serum level, triglyceride (TG) level. The mean blood level of Vitamin B12 level was 448.92±156.75pg/mL among all participants. A Vitamin B12 deficiency frequency of above 6% was seen among diabetic patients. In regression analysis, a model was defined based on which age, gender, diabetes and Metformin used had a significant effect on serum levels of vitamin B-12. Also, none of the variables gender, age, duration of treatment, metformin quantity, weight and BMI was associated with a deficiency of vitamin B12.

Conclusion: In the diabetic group, the prevalence of Vitamin B12 deficiency was more than6%. It was also seen that age, gender, diabetes mellitus, metformin intake and metformin content had a significant effect on serum B12 levels. Therefore, measurement of serum levels of vitamin B12 in metformin-treated subjects is recommended.

Keywords: Serum level; vitamin B12; type 2 diabetes; metformin.

1. INTRODUCTION

According to most of the guidelines, Metformin is the most common treatment for diabetic patients around the world today [1-3]. This drug has beneficial effects on carbohydrate metabolism, weight loss and vascular protection [2]. In contrast, it may lead to serious side-effects such as anemia [4], which may be due to vitamin B12 deficiency [5].

Vitamin B12 plays a vital role in human health. It is effective in the functioning of the nervous system and the brain and is also essential for accurate blood supply. Also, its deficiency improves renal complications among diabetic patients [6]. In addition, its deficiency and elevated blood homocysteine can cause cardiovascular disease through its effect on the metabolism of homocysteine [7].

Studies have reported a 10 to 20% decrease in plasma B12 levels among metformin-treated diabetic patients. However, despite the conducted studies, the effect of metformin on vitamin B12 levels has not been well understood [8]. Also, the relationship between the cumulative effect of metformin consumption and exacerbation of peripheral neuropathy and vitamin B12 levels has been reported [7].

On the other hand, Vitamin B12 deficiency in adults causes nonspecific clinical symptoms such as fatigue, anorexia, blood changes (megaloblastic anemia), neurological symptoms (polyneuropathy, and ataxia) and psychiatric disorders such as depression [9]. In addition, cardiovascular changes associated with increased blood homocysteine have been reported in studies [10].

However, in some studies, metformin has been identified as a risk factor for vitamin B12 deficiency and the importance of measuring vitamin B12 is emphasized [11]. There is still some doubt as to the true deficiency of Vitamin B12. In a study on mice treated with metformin, increased accumulation of vitamin B12 was seen in the liver and kidney [12].

Diagnosis and treatment of advanced cases of Vitamin B12 deficiency in people treated with long-term metformin can help to reduce complications and health costs. Studies report that evaluation of Vitamin B12 among elderly people treated with metformin is low, so that in 50% of patients under treatment by metformin for above 10 years, vitamin B12 levels is never measured [13]. Additionally, although vitamin B12 deficiency can make neuropathy worse among metformin-treated diabetic patients, it still needs further research to determine the level of vitamin B12 required to measure vitamin B12 levels among these patients [14].

Given the importance of vitamin B12 and the use of metformin as one of the main drugs for the

treatment of common type 2 diabetes, we aimed to help better understanding the complications of this treatment by examining the relationship between vitamin B12 and the use of metformin.

2. METHODS

Present study was designed and implemented as a cross-sectional work with control group. The statistical population consisted of the diabetic patients referred to Specialized Clinic of Taban and Rasoul-e-Akram Hospital in 2016.

2.1 Procedure

A total number of type 2 diabetic patients was 306(healthy= 150 and diabetics=156) treated with metformin who referred to Taban Clinic and Clinic of Rasoul-e-Akram Hospital affiliated to Iran University of Medical Sciences in 2016 were enrolled in the study. First, such information as age, gender, duration and amount of medication, Body Mass Index (BMI), High-Density Lipoprotein (HDL), Low Density lipoprotein (LDL), TG and CHOl was obtained through patient's medical history and then was filled in the questionnaire.

Patients' blood samples were taken at 8-10am and then sent to the laboratory for analysis within 30 minutes. Then, the serum levels of patients' vitamin B12 were measured using a Cobas e 602 module through Electro Chemilumin Escence Immunoassay (ECLIA). A serum level of 211-946 pmol/L or 156-698 pg/mL is considered as normal and the higher or lower values are taken as abnormal into consideration.

2.2 Exclusion Criteria

Suffering from type 1 diabetes, pernicious anemia, renal impairment (Creatine above 1.7 in men and above 1.5 in women) and severe medical conditions such as infection, sepsis, malignancies, cirrhosis of the liver, heart failure or chiral failure as well as pregnancy.

2.3 Data Analysis

Descriptive and inferential statistical methods were used for data analysis. Mean, standard deviation, and dispersion indices including maximum, minimum, and standard deviation were used to describe quantitative variables and frequency report (percentage) was employed for qualitative variables. For statistical analysis, chi-square test, t-test and ANOVA test were used in the platform of SPSS Software Ver. 20. Finally, the p-value<0.05 was considered statistically significant. In addition, Levene's test was used for measurement of variances equality.

3. RESULTS

Given the results, of 306 participants in present study, 120 ones were females (39.2%) and the rest 186 subjects (60.8%) were males. Also, 150 ones (49%) were healthy and 156 ones (51%) were suffering from diabetes Miletus.

According to Table 1, it is noteworthy to mention that of the diabetic patient participants, 75 ones were females and 81 ones were males and of the healthy subjects, 45 and 105 ones were males, indicating a significant statistical relationship between suffering from diabetes and gender (P=0.001).

Besides, according to results from questionnaire and referring to patients' medical history, the information about age, height, weight, duration of suffering from diabetes, duration of using metformin, metformin dosage, and Vitamin B12 content, Cholesterol, LDL, HDL, BMI and Triglyceride was recorded and then their mean values and standard deviation as well as median and mode were calculated (See Table 2).

Table 1. Comparison of healthy participants and patients in terms of their gender

Group	Gender		Total
	Female	Male	
Healthy	45	105	150
Patients	75	81	156
Total	120	186	306

Table 2. Descriptive statistics of the participants

No.	Variable	Mean± Standard Deviation	Median	Mode
1	Age	42.84±10.90	44	45
2	Height	166.51± 9.47	167	169
3	Weight	70.87± 11.67	71	76
4	Duration of suffering from diabetes	8.72±4.55	8	6
5	Duration of using metformin	8.36±4.05	8	8
6	Metformin dosage	1612.18±519.22	1500	1000
7	Vitamin B12 content (ng/mL)	448.92±156.75	485	440
8	Cholesterol (mg/dL)	182.92±39.45	180	149
9	LDL(mg/dL)	107.77±25.69	105.40	75.5
10	HDL (mg/dL)	41.11±11.51	38.5	35.5
11	BMI (kg/m^2)	25.67±18.84	25.56	25.10
12	Triglyceride (mg/dL)	160.75±89.37	139	73

BMI: Body Mass Index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Table 3. Comparison of males and females in terms of duration of suffering from diabetes and duration of metformin use

	Gender			
	Female 75	**Male 81**	**Levene's test for measurement of variances equality**	**P-Value**
Duration of suffering from diabetes	8.88±4.95	8.57±4.18	0.069	0.671
Duration of metformin use	8.33±4.256	3.87±8.38	0.258	0.940

Table 4. Comparison of patients and healthy individuals in terms of quantitative variables

Variables	Health status		P-Value	Levene's Test for Equality of Variances
	healthy	**Patient**		
Number of participants	**150**	**156**		
Age	38.91±11.060	46.62±9.33	0.000	0.004
Height	169.43±7.828	163.70±10.08	0.000	0.001
Weight	68.95±12.010	72.72±11.07	0.005	0.180
BMI (kg/m^2)	24.08±4.04	27.17±4.07	0.000	0.703
Serum level of vitamin B12 (ng/mL)	561.46±74.66	341.44±138.09	0.000	0.000
Cholesterol (mg/dL)	173.66±36.27	191.83±40.43	0.000	0.299
LDL(mg/dL)	102.28±24.09	113.06±26.14	0.000	0.262
HDL(mg/dL)	73.32±25.5	77.31±33.5	0.001	0.000

As it can be seen from Table 2, the mean age of participants was 42.84±10.90. Also, the mean height and weight of participants were 166.51±9.47 and 80.87±11.67, respectively. The duration of suffering from diabetes among patients was 8.72±4.55 years. In addition, the mean duration of using metformin was 8.36±4.05 years and the corresponding mean dosage was 1612.18±519.22 mg. Blood level was also 448.92±156.75. The cholesterol content among all participants was 182.92±39.45. The mean HDL and LDL contents among the subjects were 107.77±25.69 and 41.11±11.51, respectively. The mean BMI was also calculated to be

25.67±4.34. Lastly, the mean triglyceride content was 160.75±89.37.

Of the metformin-treated patients, only one female was treated by 500mg metformin, and among the recipients of 1000mg metformin per day, there were 31 females and 17 males. 18 females and 23 males received 1500 mg metformin daily and 15 females and 32 males were receiving 2000mg metformin. Also, 10 females and 9 males of patients daily received 2500mg metformin, indicating a significant relationship between metformin use and gender (P=0.02).

The mean duration of metformin use among patients was 8.33±4.256 and 3.87±8.38 for females and males, respectively. According to inequality of variances (0.258), no significant relationship was found between duration of metformin use and gender (p=0.940) (Table 3).

Following to comparison of type 2 diabetes mellitus and healthy individuals in terms of quantitative variables including age, height, weight, BMI, serum level of vitamin B12, serum cholesterol level, LDL serum level, and HDL serum level, a statistically significant difference was seen (Table 4).

The participants were divided into 5 groups in terms of metformin dosage (0, 1000, 1500, 2000, 2500 mg/day). These groups were compared according to the variables weight, BMI, Cholesterol and LDL by ANOVA test. The results can be seen in Table 5.

All variables of Table 5 were compared in terms of variances equality, where all P-values were greater than 0.05.

Since the inequalities of variances on age, height, serum level of vitamin B12, HDL and triglyceride were all proved to be significant in Levene's test, Kruskal-Wallis test was used to investigate the relationship of different groups based on metformin use with the mentioned variables. The results can be seen in Table 6 where the mean rank and p-value are obtained from Kruskal-Wallis test.

In regression analysis, a model was defined whose information can be found in Fig. 1 (exported from SPSS Software). As it can be seen, age, gender (sex), suffering from diabetes, metformin use and amount of used metformin (dosage) have a significant impact on serum level of Vitamin B12.

Table 5. Comparison of different groups of metformin consumers based on weight, BMI, cholesterol and LDL

	Metformin dosage					P-Value
	0	**1000**	**1500**	**2000**	**2500**	
Number of participants	**150**	**49**	**41**	**47**	**19**	
Weight	68.95±12.01	73.14±11.48	73.95±9.12	71.51±11.10	72.74±14.05	0.067
BMI (kg/m^2)	24.08±4.04	27.64±3.86	26.66±3.86	26.91±4.35	27.84±5.16	0.002
Cholesterol (mg/dL)	173.66±36.27	191.04±41.78	190.65±31.99	192.05±47.07	195.84±38.28	0.005
LDL (mg/dL)	102.28±24.09	114.39±27.58	109.61±22.22	114.07±29.16	114.57±23.40	0.000

Table 6. Results of Kruskal-Wallis test and p-value for the quantitative variables with unequal variances among the groups treated by metformin

	Metformin dosage					P-Value
	0	**1000**	**1500**	**2000**	**2500**	
Number of participants	**150**	**49**	**41**	**47**	**19**	
Serum level of Vitamin B12 (ng/mL)	218.73	155.67	95.41	46.26	18.92	0.000
Age	123.18	111.09	162.87	239.59	269.08	0.000
Height	182.11	113.29	144.98	128.35	111.95	0.000
HDL (mg/dL)	167.69	141.80	125.99	137.77	169.97	0.029
Triglyceride (mg/dL)	132.16	162.35	192.26	180.51	148.71	0.000

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	0.913[a]	0.833	0.830	64.584	0.833	298.367	5	299	0.000

a. Predictors: (Constant), The amount of Metformin, Sex, Age(year), DM.Status, For Metformin

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	579.022	25.402		22.794	.000	529.032	629.012
	For Metformin	-11.140	2.278	-.362	-4.890	.000	15.623	-6.657
	Age(year)	-2.679	.457	-.186	-5.865	.000	-3.577	-1.780
	Sex	20.701	8.070	.065	2.565	.011	4.819	36.583
	DM.Status	51.668	9.294	.330	5.559	.000	33.379	69.957
	The amount of Metformin	-.128	.016	-.724	-8.031	.000	-.159	.096

a. Dependent Variable: V12

Fig. 1. The defined regression analysis in SPSS software

Table 7. Frequency for vitamin B12 (ng/mL) deficiency (with the values lower than 156)

		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	.00	10	3.3	3.3	3.3
	1.00	296	96.7	96.7	100.0
	Total	306	100.0	100.0	

Finally, in the group of diabetic patients, a frequency of above 6% for Vitamin B12 deficiency (with the values lower than 156) was seen. In addition, all individuals having B12 deficiency were in the patients group.

4. DISCUSSION

We observed that there was a statistically significant difference between the patients with type 2 diabetes mellitus and healthy individuals in terms of quantitative variables. This can be explained by the metabolic effects of diabetes.

Similarly, in Camarero et al. study [15], Vitamin B12 deficiency was reported to be 9.1%, while 49% of these subjects were below 65 years old and a significant relationship (P<.001) was seen between Vitamin B12 deficiency and age, dementia, changes in blood cell count, memory, metformin use and proton pump inhibitors (PPI) (P=0.007).

Khodabandehloo et al. [16] reported that the mean blood level of vitamin B12 in all subjects was 448.92±156.75 ng/mL. In their diabetic group, the prevalence of B12 deficiency was seen to be above 6%. In a study conducted among an Iranian population, prevalence of Vitamin B12 deficiency was demonstrated to be among the elderly people [17]. Another cross-sectional study_conducted in Iran reports a B12 Vitamin deficiency higher than the average of other studies [17].

Referring to National Health and Nutrition Examination Survey (NHANES) in 1999-2006, a prevalence of 5.8% was reported for vitamin B12 deficiency among diabetics treated with metformin; while, a rate of 16.2% was reported for slight Vitamin B12 deficiency [18]. Also, in Braza et al., study [19], an investigation was undertaken on 76 patients, where 18.6% of them were Vitamin B12 deficient and 22.3% had the vitamin B12 levels lower than normal, while totally 40% of patients had lower than normal levels of Vitamin B12.

In Metaxas et al., study [20], Vitamin B12 deficiency was considered to be below 200 ng/mL based on which a frequency of 34% was reported. Also, the relationship between Vitamin B12 serum level and metformin has been

demonstrated formerly [21] which is in line with findings of present study.

Besides, in a new study conducted on mice, accumulation of Vitamin B12 is reported in liver and kidney, indicating low vitamin B12 serum level [12]. Another justification for B12 deficiency following metformin use is its effect on calcium-dependent membrane function in the terminal part of the ileum [22].

In our regression analysis, a model was defined based on which age, gender (sex), suffering from diabetes, metformin use and amount of used metformin (dosage) have a significant impact on serum level of Vitamin B12. Here, the least and most duration of metformin was 2 and 18 years, respectively, with a mean of 8 years. However, according to former studies, it was more than proposed durations for onset of metformin effectiveness on serum level of Vitamin B12. In the study of Lohmann [23], the level of Vitamin B12 significantly decreased following to metformin intake compared to placebo. In contrast, in our results no relationship was seen between metformin use duration and Vitamin B12. This finding is in line with Gupta et al., [22] where an inverse relationship between metformin use duration and Vitamin B12 level. Similarly, Metaxas et al., [20] report diabetes as effective factors on Vitamin B12 level, which is parallel with results of present study.

On the other hand, in present study, the mean metformin dosage was 1612.18±819.22mg. In doing so, such studies as [24-25] report the relationship between metformin dosage and Vitamin B12 level.

Also, none of such variables as gender, age, duration of treatment, metformin dosage, weight and BMI were associated with deficiency of vitamin B12. This is while, the possibility of vitamin B12 deficiency followed by metformin intake is reported to be higher among elderly people [26].

5. CONCLUSION

According to a vitamin B12 deficiency frequency of above 6% among diabetic patients and also significant effectiveness of age, gender, suffering from diabetes mellitus, metformin use and metformin dosage on serum B12 levels, measurement of serum levels of vitamin B12 in metformin-treated subjects is recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53717