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# Prevalence of Vitamin B12 Deficiency in Type 2 Diabetes Mellitus Patients on Metformin

**Dr. Ali Fadhil Mohammed, M.B.Ch.B., F.I.C.M.S (Internal Medicine ) / Thi-Qar Health Directate**
**Dr. Dheyaa Khalaf Al-Omer, M.B.Ch.B., C.A.B.M ( Internal Medicine), M.A.C.P. / Department of Medicine / Thi-Qar College of Dentistry**

## Abstract

**Background:** Diabetes Mellitus is the commonest endocrine disease and metformin is the commonest oral anti diabetic agent prescribed. B12 deficiency is one of the side effects of metformin due to the effect on calcium-dependent membrane action in the terminal ileum leading to malabsorption of vitamin B12.

**Aim of study**: for determination of the prevalence and association of Vitamin B12 deficiency in patients with type 2 diabetes mellitus managed with metformin.

## Methods:

This case control study took place in the department of medicine, Alhussein teaching hospital in Nassiriyah city in Iraq .We selected 43 patients with type 2 diabetes mellitus on metformin for at least 12 months and 42 patients were taken as control. Patients with vitamin B12 levels of less than 297 pg/mL or methyl malonic acid (MMA) more than 47 ng/mL were considered B12 deficient.

## Results:

serum B12 level was low in 17 out of 43 patients (39.5%) among cases, and 8 out of 42 patients (19%) among controls . Mean age was 50 (±12.86) years in the control and 52 (±12.12) years in the case group. Control group composed of 23 males and 19 females, while case group composed of 17 males and 26 females. Duration of DM was 5.1 (±4.5) years for control and 6.1 (±3.75) years for cases. HbA1c % was 8.44 (±2) for control and 8.25 (±1.65) for case . Vitamin B12 level was 349 (±99) pg/ml for control and 343 (±124) pg/ml for cases. B12 deficiency correlated significantly with the duration and dose of metformin with (P.value=0.001and 0.002) respectively.

**Conclusion:** we found that there was a high prevalence of vitamin B12 deficiency among patients managed on metformin with significant effect of duration and dose of metformin use on B12 levels. This should be considered in patients treated with metformin.

**Key words** : Diabetes mellitus , metformin , vitamin d deficiency .

## Introduction

Diabetes mellitus (DM) is the most common endocrine disorder characterized by increased fasting or post prandial plasma glucose levels more than upper limits during oral glucose tolerance test or random plasma measures as defined by the criteria for the diagnosis of DM [1,3,4]. Diabetes and it's complications is the fifth leading cause of death worldwide [2].

According to the pathogenic process that lead to hyperglycemia, diabetes mellitus is classified into; type 1 DM, type 2 DM that is the most common type of DM which account for about 85% of cases , gestational DM and other specific types of DM [3].

The goals of therapy for type 2 DM should include good glycemic control, treatment of associated conditions and screening for and management of complications. Type 2 diabetes management should begin with medical nutrition therapy (MNT); an exercise regimen to increase insulin sensitivity and promote weight loss should also be instituted. Pharmacologic agents to the management of type 2 diabetes mellitus include oral glucose-lowering agents, insulin, and other agents that improve glucose control or combination of these drugs , in the absence of contraindications, metformin is considered the first choice for oral treatment of type 2 diabetes [3] . one of the side effects of metformin is vitamin B12 deficiency . The decrement in vitamin B12 absorption and levels following metformin use typically starts as early as the forth month. Clinically overt features of vitamin B12 deficiency manifest by about 5 years due to the large body stores predominantly in the liver. The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with type 2 diabetes mellitus include alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels and interaction with the cubilin endocytic receptor[1-4] .

Treatment of vitamin B12 deficiency does not differ irrespective of the cause.

## Aim Of Study

The aim of this study is to determine prevalence and association of vitamin B12 deficiency in patients with type 2 diabetes mellitus treated with metformin in Nassiriyah city.

## Patients And Method

Our study was a case-control study performed in Al-Hussein teaching hospital in Nassiriyah city, over a period of 9 months between 1st of May 2018 to 31th of January 2019. We selected 85 patients with type 2 diabetes mellitus diagnosed within the past one year, aged between 20 and 69 years. 43 of them were on metformin for at least 1 year and considered as case group, While 42 one of them were patients with type 2 diabetes mellitus not on metformin who were considered as the control group . Patient were excluded from our study if they had anemia, renal impairment, chronic liver disease, malabsorption, gastric surgery, pregnancy or if the patients were vegetarian or on proton pump inhibitors (PPI), H2 receptor blockers or B12 supplements. Tested patients were asked to attend the laboratory. Venous blood samples were taken from them by full aseptic technique, samples from each patient separated into two tubes and analysed for Random blood glucose, blood urea, serum creatinine and Hemoglobin A1c were done by Cobas c111 , while complete blood count was done by Mindray B 5000 and B12 level was done by chemi-luminesent immunoassay ( CLIA ) and methyl malonic acid was done by enzyme-linked immunosorbent assay

( ELISA ) . The results were verified by expert laboratory team. Data collected for each patient included age, gender, duration of diabetes mellitus, dose and duration of metformin use. Data analysis was done by statistical package for social sciences (SPSS) version 22 for percentage and frequencies, Chi-Square test or fisher exact test were used for study association , correlation between variables was analyzed using Pearson equation, P value of less than 0.05 was considered statistically significant. The normal values for B12 is between (279 - 996 pg/mL ), for MMA is less than 47 ng/mL and HbA1c of less than 7 % was considered controlled diabetic patients.

## Results

**Table (1) Demographic characteristics of study group**

| Demographics | Control Group | Case Group | P Value |
|---|---|---|---|
| Age (Yr) Mean (SD) | 50.488± (12.86) | 52.139 ±(12.12) | 0.542 |
| Patient's Number (%) | 42 (49.4 %) | 43 (50.6 %) | 0.160 |
| Male Number (%) | 23 (27.1 %) | 17 ( 20 %) | 0.430 |
| Female Number (%) | 19 (22.4 %) | 26 (30.6 %) | 0.370 |
| DM Duration (Yr) Mean (SD) | 5.1905± (4.5) | 6.1279± (3.75) | 0.614 |
| Hba1c % Mean (SD) | 8.4438 ±(2.03) | 8.2553± (1.65) | 0.390 |
| Vit. B12 Level Pg/Ml Mean (SD) | 349.83± (99.58 ) | 343.26± (124.14) | 0.038 |

Table one showed that; the number of patients included in our study were 85 patients , 42 of them were within control group which are the diabetic patients not on metformin, while 43 of them were case were diabetic patients on metformin. Mean age is 50.488± (12.86) years for control and 52.139 ± (12.12) years for case group. Control group composed of 23 males and 19 females, While the case group composed of 17 males and 26 females. Duration of DM was 5.1905± (4.5) years for control and 6.1279± (3.75) years for cases. HbA1c % was 8.4438 ±(2.03) for control and 8.2553± (1.65). Vit. B12 level was 349.83± (99.58) pg/mL for control and 343.26± (124.14) pg/mL for cases .

**Table (2) Demographic characteristics of patient on metformin**

| Demographics | Deficient Vitamin B12 (Less Than 279 Ng/Ml ) | Normal Vitamin B12 Level (Equal Or More Than 279 Ng/Ml) | P Value |
|---|---|---|---|
| Age (Yr) Mean (SD) | 50.13± (11.98) | 52± (12.7) | 0.523 |
| Patient Number (%) | 17 (39.5 %) | 26 (60.5 %) | 0.570 |
| Male Number (%) | 11 (25.6 %) | 6 (14 %) | 0.180 |
| Female Number (%) | 6 (14 %) | 20 (46.5 %) | 0.250 |
| DM Duration (Yr) Mean (SD) | 5.58± (3.65) | 3± (2.19) | 0.663 |
| Hba1c % Mean (SD) | 8.347± (1.71) | 8.349 ±(1.91) | 0.437 |
| Vit. B12 Level Pg/Ml Mean (SD) | 236.2± (30.47) | 413.2 ±(111.4) | 0.038 |
| Metformin Duration (Yr) Mean (SD) | 4.617 ±(3.34) | 3±(2.19) | 0.001 |
| Metformin Dose Mg/Day Mean (SD) | 1032± (561.51) | 682± (330.93) | 0.002 |

Table two showed that; the number of cases with metformin were 43 patients including 17 with low B12 level, defined as a B12 level less than 279 pg/mL
, while 26 of them were case which were within normal B12 level . Mean age is 50.13± (11.98) years for low B12 level group and 52± (12.7) years for the normal B12 level group. Low B12 group composed of 11 males and 6 females, while normal B12 group composed of 6 males and 20 females. Duration of DM was 5.58± (3.65) years for low B12 level group and 3±(2.19) years for normal B12 level group. HbA1c % was 8.347± (1.71) for low B12 level group and 8.349 ±(1.91) for normal B12 level group. Vitamin B12 level was 236.2± (30.47 pg/mL for low B12 level group and 682± (330.93) pg/mL for cases. Duration of metformin used was 4.617
±(3.34) and 3±(2.19) years for low and normal B12 level respectively. Dose of metformin in mg/day was 1032± (561.51) and 682± (330.93) for low and normal B12 level respectively. P value was significant for duration and dose of metformin. Value of 0.001 for the duration of metformin and a value of 0.002 for the dose of metformin .

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**Table (3) B12 distribution according to study group**

| | | | B12 | | | P.value |
|---|---|---|---|---|---|---|
| | | | Low Less than 279 ng/mL | Normal Equal or More than 279 ng/mL | Total | |
| Type | Control | Count | 8 | 34 | 42 | |
| | | % | 19.0% | 81.0% | | |
| | Case | Count | 17 | 26 | 43 | |
| | | % | 39.5% | 60.5% | | |
| Total | | Count | 25 | 60 | 85 | 0.038 |
| | | % | 29.4% | 70.6% | 100.0% | |

B12 level considered low when it is below 279 pg/mL [3] .
The table three showed that the number of all patients included in our study were 85 patients. 42 of them were considered as the control group who were diabetic patients not on metformin, while 43 of them were diabetic patients on metformin. Regarding the control group we found that (8 out of 42) patient were had a low B12 level (19%) while (34 out of 42) patients had normal B12 levels (81%). With regards to the case group we found that (17 out of 43) patient had a low B12 level that represent 39.5%, while (26 out of 43) patients were within normal B12 level that represent 60.5 %. It showed that there is significant correlation between both study groups with P value (0.038)

**Table (4) MMA distribution according to study group**

| | | | MMA | | | P. value |
|---|---|---|---|---|---|---|
| | | | (High) More than 47ng/ml | (Normal) 47ng/ml or less | Total | |
| Type | Control | Count | 8 | 34 | 42 | |
| | | % | 19.0% | 81.0% | 100.0% | |
| | Case | Count | 14 | 29 | 43 | |
| | | % | 32.6% | 67.4% | 100.0% | |
| Total | | Count | 22 | 63 | 85 | 0.155 |
| | | % | 25.9% | 74.1% | 100.0% | |

MMA level considered high when it exceed 47 ng/ml.

The table four showed that the number of all patients included in our study were 85 patients, 42 of whom were considered as the control group whose were the diabetic patients not on metformin. 43 of them were diabetic patients on metformin. Regarding the control group we found that (8 out of 42) patients had high MMA level (19%), while (34 out of 42) patients were within normal MMA levels (81%). Regarding the case group we found that (14 out of 43) patient were with high MMA levels representing 32.6% of the sample, while (29 out of 43) patients were within normal MMA levels representing 67.4 %. It showed that there is no significant correlation between both study groups with a P value (0.155)

**Table (5) B12 distribution according to duration of DM in years within the study groups**

| Type | | | | B12 | | | |
|---|---|---|---|---|---|---|---|
| | | | | Low | Normal2 | Total20 | |
| control | DM duration in years | Less than 5 | Count | | 18 | 47.6% | |
| | | | % | 4.8% | 42.9% | 22 | |
| | | Equal or more than 5 | Count | 6 | 16 | 52.4% | |
| | | | % | 14.3% | 38.1% | 42 | |
| | Total | | Count | 8 | 34 | 100.0% | 0.155 |
| | | | % | 19.0% | 81.0% | 21 | |
| Case | DM duration in years | Less than 5 | Count | 9 | 12 | 48.8% | |
| | | | % | 20.9% | 27.9% | 22 | |
| | | Equal or more than 5 | Count | 8 | 14 | 51.2% | |
| | | Total | % | 18.6% | 32.6% | 43 | 0.663 |
| | | | Count | 17 | 26 | 100.0% | |
| | | | % | 39.5% | 60.5% | 41 | |
| Total | DM durationin years | Less than 5 | Count | 11 | 30 | 48.2% | |
| | | | % | 12.9% | 35.3% | 44 | |
| | | Equal or more than 5 | Count | 14 | 30 | 51.8% | |
| | | | % | 16.5% | 35.3% | 85 | |
| | Total | | Count | 25 | 60 | 100.0% | 0.614 |
| | | | % | 29.4% | 70.6% | | |

The Low and Normal -B12 groups accounted for 19% and 81% of the control group respectively while the Low and Normal accounted for 39.5% and 60.5% of the case group respectively.

The (<5), (≥5) years DM duration groups accounted for 47.6%, 52.4% respectively of the control group and 48.8%, 51.2% respectively of the case group.

The (<5), (≥5) DM duration groups accounted for 48.2% and 51.8% for all patients in both study groups respectively.

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**Table (6) B12 distribution according to duration of metformin use in years within the case group**

| Type | | | | B12 | | Total | P value |
|---|---|---|---|---|---|---|---|
| | | | | Low7 | Normal23 | | |
| Case of met. | Duration Inyears | Less than5 Equal or | Count | 16.3% | 53.5% | 30 | |
| | | | % | 10 | 3 | 69.8% | |
| | | more than 5 | Count | | | 13 | |
| | | | % | 23.3% | 7.0% | 30.2% | |
| | Total | | Count | 17 | 26 | 43 | 0.001 |
| | | | % | 39.5% | 60.5% | 100.0% | |

Patient on metformin was divided into 2 groups according to the duration of useof metformin, less than 5 years and equal to or more than 1000 mg/day.

The table above showed that there is significant correlation with the duration of metformin use with a P.value 0.001.

**Table (7) B12 distribution according to dose of metformin use within the case group**

| Type | | | | B12 | | Total | P value |
|---|---|---|---|---|---|---|---|
| | | | | Low5 | Normal | | |
| Case | Dose of met. In mg/day | Less than 1000 mg/day | Count | 11.6% | 20 | 25 | |
| | | | % | 12 | 46.5% | 58.1% | |
| | | Equal or more than 1000 mg/day Total | Count | | 6 | 18 | |
| | | | % | 27.9% | 14.0% | 41.9% | |
| | | | Count | 17 | 26 | 43 | 0.002 |
| | | | % | 39.5% | 60.5% | 100.0% | |

Patient on metformin were divided into 2 groups according to the dose, less than1000 mg/day and equal to or more than 1000 mg/day.

The table above showed that there is significant correlation with the dose ofmetformin use with P.value 0.002

## Discussion

One of the well-known side effects of metformin use is vitamin B12 deficiency. This study was performed to assess the prevalence of vitamin B12 deficiency in diabetic patients with metformin

in comparison with diabetic patients who was not use metformin. In this study, found that the percent of B12 deficiency among patients on metformin was 39.5 % , compared to 19% of B12 deficiency among diabetic patients with not used metformin . This means there is significant correlation between metformin use and B12 deficiency, with a P. value 0.038. We regarded either 279 pg/mL or MMA level higher than 47 ng/mL as the cut off point for considering the patients B12 deficient [3,5-9]. A similar study was performed in Pakistan in 2012 by Iftikhar et al , their demographic data and exclusion criteria were the same as our study, they found that, the percent of deficiency among patients on metformin was 31% while in patients without metformin, the percent of vitamin B12 deficiency was 9% , so this showed significant correlation between metformin use and vitamin B12 deficiency with P. value 0.03, the cut off point for considering B12 deficient in Iftikhar et al study was 150 pg/mL .This may explain the higher percent of B12 deficiency among cases and controls of our study, when compared to their results [10] .

Another study was done in Pakistan by Hasan et al in 2017. They found that 27.5% had deficiency among patients on metformin with deficient levels and 9% among the patients without metformin. The cut off value for considering B12 deficiency in their study was 298 pg/mL [11].

In a study done in Brazil in 2009 by nervo et al, they found that vitamin B12 was deficient in 6.9% of patients on metformin when considering the cut off value was 169 pg/mL as compared to 36.8% of patients with B12 deficiency when considering the cut off value as 338 pg/mL [12].

Another study was done by Pflipson et al in 2009, also showed that the percentage of B12 deficiency among patients with metformin was 22% and they used 100 pg/ml , as a cut off point for considering B12 deficient [13] .

From all these studies, we found that, there is no fixed value for determining the normal value of B12 level and in addition to that the measurement of serum B12 level is not a specific indicator for B12 deficiency. We need to consider other tests to reach a more accurate result. Although we need to search for the cause of vitamin B12 deficiency, we could not exclude them in our study such as pernicious anemia, tapeworm infestation, bacterial overgrowth, congenital or inherited causes of B12 deficiency.

Other reason that may explain this high percent of B12 deficiency among both study groups may belong to non-prescriptive use of proton pump inhibitors (PPI) or H2-receptor antagonists, although we included it in an exclusion criteria of our study, but many of the patients still use these drugs without prescription by the physician and ignored their usage for these drugs when we asked them about drug history.

Regarding the duration of metformin, it is significantly correlated with B12 deficiency, with P. Value 0.001 and mean duration of metformin use 4.5 ± 3 years, These results were comparable with results of other studies like Iftikhar et al , with P.value 0.001 and mean duration of metformin use around 4 years, and a study by Nervo et al with P. value (0.048) and a study of Ahmed et al , with P. value 0.015 and mean duration of DM around 8 years .

Regarding the dose of metformin, we found significant correlation with B12 deficiency, with a P. value 0.002 and mean for metformin dose 1000 ± 500 mg daily. This result was comparable with the results of other studies. Iftikhar et al study showed P. value 0.003 with mean metformin dose around 2000 mg daily.

Pflipson et al study found that there was no significant correlation between the dose of metformin and B12 deficiency with a P. value 0.09 and mean dose of metformin around 2000 mg daily, they showed that, the higher dose of metformin may effect B12 levels due to the presence of other risk factors.

Regarding age, there was no significant correlation between age and metformin, where P. value was 0.523 and this result was comparable with the results of other studies, but we found that, higher percent of vitamin B12 deficiency was among elderly patients in age group between (60-69) years in both case and control groups and this finding was similar to the results of studies of Hasan et al , Ahmed et al , and Kang et al [14] , in these studies , they showed the same correlation between age and vitamin B12 deficiency, this result may be explained by the lack of a balanced diet in elderly patients, misuse of other medications. Elderly patients may use aspirin more than younger patients and this in turn lead to reduction of secretion of intrinsic factor or hypo-chlorhydria, and these are considereda risk factor for B12 deficiency [15].

## Conclusion

In our study, we found there is high prevalence of vitamin B12 deficiency among patients who were treated with metformin and this prevalence significantly correlated with dose and duration of metformin. This elevation is comparable with the results of other studies across the globe. The prevalence of vitamin B12 deficiency was also high in diabetic patients not on metformin when compared to the results of other studies, this should guide us to search for the cause of this deficiency. The high prevalence vitamin B12 deficiency in the control group needs more investigations.

## References

1. Thomas N. Diabetes mellitus: The responsibility of the generalist and specialist. Current Medical Issues. 2017;15(3):165.
2. Ralston S, Penman I, Strachan M, Hobson R. Davidson's Principles and Practice of Medicine E-Book. 23rd ed. Philadelphia: Elsevier; 2018.
3. Loscalzo J, Wiener C, Brown C, Houston B, Kasper D, Fauci A et al. Harrison's principles of internal medicine. 19th ed. New York: McGraw-Hill Education; 2017.
4. Kumar R, Nandhini L, Kamalanathan S, Sahoo J, Vivekanadan M. Evidence for current diagnostic criteria of diabetes mellitus. World Journal of Diabetes. 2016;7(17):396.
5. Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. BMJ. 2014;349(sep04 1):g5226-g5226.
6. Provan A, Dokal I, Vos J, Baglin T. Oxford handbook of clinical haematology. Oxford: Oxford University Press; 2016
7. Mineva E, Zhang M, Rabinowitz D, Phinney K, Pfeiffer C. An LC-MS/MS method for serum methylmalonic acid suitable for monitoring vitamin B12 status in population surveys.

Analytical and Bioanalytical Chemistry. 2014;407(11):2955-2964.
8. Braschi C, Doucette J, Chari A. Characteristics of Vitamin B12 Deficiency in Patients With Plasma Cell Disorders. Clinical Lymphoma Myeloma and Leukemia. 2017;17(12):e65-e69.
9. Sobczyńska-Malefora A, Gorska R, Pelisser M, Ruwona P, Witchlow B, Harrington D. An audit of holotranscobalamin ("Active" B12) and methylmalonic acid assays for the assessment of vitamin B12 status: Application in a mixed patient population. Clinical Biochemistry. 2014;47(1-2):82-86.
10. Iftikhar R, kamran S, Qadir A, Iqbal Z, Usman H. Prevalence of Vitamin B12 deficinecy in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. Pan African Medical Journal. 2013;16.
11. T. S. Vitamin B12 Deficiency in Metformin Treated type 2 Diabetic Patients and Factors Associated with it: A Cross-Sectional Study. Journal of Medical Science And clinical Research. 2018;6(6).
12. Nervo M, Lubini A, Raimundo F, Moreira Faulhaber G, Leite C, Fischer L et al. VitaminB12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. Revista da Associação Médica Brasileira. 2011;57(1):46-49.
13. Khan A, Shafiq` I, Hassan Shah M. Prevalence of Vitamin B12 Deficiency in Patients with Type II Diabetes Mellitus on Metformin: A Study from Khyber Pakhtunkhwa. Cureus. 2017;.
14. Kang D, Yun J, Ko S, Lim T, Ahn Y, Park Y et al. Higher Prevalence of Metformin-Induced Vitamin B12 Deficiency in Sulfonylurea Combination Compared with Insulin Combination in Patients with Type 2 Diabetes: A Cross-Sectional Study. PLoS ONE. 2014;9(10)
15. Ham A, Enneman A, van Dijk S, Oliai Araghi S, Swart K, Sohl E et al. Associations Between Medication Use and Homocysteine Levels in an Older Population, and Potential Mediation by Vitamin B12 and Folate: Data from the B-PROOF Study. Drugs & Aging. 2014;31(8):611-621.