HAEMATOLOGICAL PARAMETERS AND VITAMIN B12 LEVELS IN TYPE II DIABETIC PATIENTS ON METFORMIN- A PROSPECTIVE CASE CONTROL STUDY

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ABSTRACT

BACKGROUND

Metformin is the cornerstone medication in the management of type 2 diabetes mellitus (T2DM) with estimates that it is routinely prescribed to 120 million type 2 diabetes patients around the world. Preceding studies say long term use of metformin in T2DM lowers vitamin-B12 levels and its clinical manifestations can be misdiagnosed as diabetic peripheral neuropathy. The aim of this study is to correlate haematological parameters with estimated serum Vit. B12 levels in T2DM patients on long term metformin in patient subjects and healthy volunteers.

MATERIALS AND METHODS

A total of 34 T2DM patients (cases) and 34 healthy volunteers (controls) were enrolled in the study from the out-patient department and in-patient wards of RVM Hospital, for over a period of 6 months. Serum vitamin-B12, HbA1c and haematological parameters were measured.

RESULTS

The clinical characteristics and serum vitamin-B12 levels of the study population were compared using chi-square cross tabulation. Vitamin-B12 deficiency was higher in T2DM patients taking daily dose of metformin >1000 mg/day. Hematological parameters estimation and peripheral smear study was done in all cases to identify macrocytic anaemia and hyper segmented polymorphs.

CONCLUSION

The prevalence of vitamin-B12 deficiency in T2DM patients was found to be high in our study population when compared to control subjects. Peripheral smear study revealed macrocytic anaemia in 22.7% and hyper-segmented polymorphs in 50% of cases with B12 deficiency. Considering the regular screening of vitamin-B12 as cost factor, the peripheral smear can be done frequently in T2DM patients on long term metformin to detect macrocytic anaemia followed by biochemical analysis periodically in a rural areas.

KEYWORDS

Peripheral smear, Hematologic parameters, Metformin, T2DM, Vitamin-B12, HbA1c.

HOW TO CITE THIS ARTICLE: Koduri VL, Nori SNS, Aditya SRKK, et al. Haematological parameters and vitamin B12 levels in type ii diabetic patients on metformin- a prospective case control study. J. Evid. Based Med. Healthc. 2018; 5(31), 2317-2323. DOI: 10.18410/jebmh/2018/478

BACKGROUND

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that is increasingly becoming a pandemic in developed and developing worlds.[1] According to the World Health Organization, the number of type-2 DM patients is expected to double within the next 25 years[2] and according to International Diabetes Federation there were over 72 million cases of diabetes in India in 2017.

Metformin is the most prescribed drug for type 2 diabetes. It is cost effective and has an excellent safety profile for most individuals. However, in the last ten-fifteen years, there was a suspicion that long term treatment with metformin caused vitamin B12 deficiency which was mistaken for diabetes induced peripheral neuropathy.

The clinician must be aware of the possibility of metformin-associated B12 deficiency in users who suffer cognitive impairment, peripheral neuropathy, sub-acute combined degeneration of the cord or anaemia. It must be taken into account that vitamin B12 deficiency leads not only

Financial or Other, Competing Interest: None.
Submission 10-07-2018, Peer Review 17-07-2018,
Acceptance 24-07-2018, Published 30-07-2018.
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DOI: 10.18410/jebmh/2018/478

to megaloblastic anaemia and neuropsychiatric disorders, but also has detrimental effects on cardiovascular health due to iatrogenic hyper-homocysteinaemia.[3]

Metformin affects calcium dependent membrane action in the uptake of B12-intrinsic factor complex by ileal cell membrane receptors. Hence the resulting B12 deficiency can be reversed by administering calcium.[4]

Vitamin B12 is involved in the optimal functioning of the hemopoietic, neuro-cognitive and vascular systems. It is involved in DNA synthesis, fatty acid metabolism and energy production.[5] Vitamin B12 plays an important role in the functioning of the brain and nervous system, in the formation of red blood cells. Its deficiency causes megaloblastic anaemia and may increase the severity of peripheral neuropathy in patients with T2DM. Vitamin B12 participates in the pathway of homocysteine (Hcy) metabolism, a reduction in vitamin B12 increases plasma concentrations of Hcy, resulting in cardiovascular disease in patients with T2DM and PCOS.[4]

Some clinical studies reported that metformin lowered vitamin B12 level, but other studies have refuted that. Consensus has not been reached on whether metformin induces vitamin B12 reduction till date.

We conducted a prospective study to assess the association between metformin treatment, vitamin B12 reduction and hematologic parameters as vit-B12 deficiency is one of the aetiological factors for megaloblastic anaemia. The aim of the study is to find the effectiveness of CBP and careful peripheral smear study in detecting macrocytic anaemia and hyper-segmented polymorphs. Hyper-segmented polymorphs in peripheral smear is an early indicator for macrocytic anaemia / vitamin B12 deficiency.[5]

Aim of the Study

The aim of this study is to correlate haematological parameters with estimated serum vit-B12 levels in T2DM patients on long term metformin in patient subjects and healthy volunteers. A total of 34 T2DM patients (cases) and 34 healthy volunteers (controls) were enrolled in the study from the out-patient department and in-patient wards of RVM Hospital, for over a period of 6 months.

MATERIALS AND METHODS

A total of 34 patients with type 2 diabetes mellitus (T2DM) and 34 healthy volunteers between the age group of 30 to 70 years were enrolled into the prospective case-control study using a convenient sampling method. All the T2DM patients were on metformin and have been on the drug for a minimum of 6 months.

Inclusion Criteria

Cases: T2DM individuals >30 years age and on Metformin > 6 months.

Controls: Non-diabetics (age and sex matched).

Exclusion Criteria

Patients with newly Diagnosed T2DM, Gastrectomy, Colectomy, liver disease, chronic kidney disease, Pernicious Anaemia, Type-I Diabetes patients, IBD and thyroid diseases were excluded from this study. Also, patients with recent intake of oral or intramuscular vitamin- B12 supplement, patients on histamine 2 receptor blocker and Vegetarians were also excluded.

Informed Consent

All the participants were enrolled into this study after obtaining an informed consent from each of the participants. Sample collection and storage.

After an overnight fasting of about 8 – 10 hours, 3 ml of venous blood was collected from each subject and dispensed in clot retrieving tubes. The samples were centrifuged at 1500 rpm for 10 minutes to obtain serum samples which were kept at -80^{0}C until analysed for vitamin-B12 level.

Determination of serum vitamin B12 level and haematological parameters

Serum vitamin B12 levels of the patients were determined using CENTOR-XP machine by chemiluminescence method and haematological parameters like were measured by Horiba 5-part Cell Analyser. Peripheral Smears were stained with Leishman's reagent and examined under microscope.

Statistical Analysis

The Serum B_{12} levels were compared with haematological parameters, HbA1c and metformin years of use by Chi-square cross tabulation. Also, the paired samples correlation and odds ratio was done. Data with gaussian distribution were presented as mean ± standard deviation. All the statistical analysis was performed using SPSS (version 25.0) and Microsoft excel.

In this study we have used values of-

- <200 pg/m/ml for definite vitamin B12 deficiency (147.6pmol/L)
- >200 to 300 pg/ml for borderline vitamin B12 deficiency. (147.6 - 221.4pmol/L)
- >300 pg/ml for normal vitamin B12 levels (221.4pmol/L)

RESULTS

The baseline characteristics of the study population are shown in Table 1. The prevalence of serum vitamin B12 deficiency and borderline deficiency in T2DM patients are 20.5% and 41.1%. As shown in table 2, 41.1% of the peripheral smears were positive for hyper-segmented neutrophils indicating the presence of megaloblastic anaemia / vit-B12 deficiency in the study cases. Peripheral smear reports of the controls were normocytic and normochromic in 70.58%.

According to table 3, vitamin-B12 deficiency in patients with 1-10 years of metformin use was seen in 83% of cases (20/24). There is a significant negative relation between age and vitamin B12 range in our study as shown in table 4, showing that increase in age decreases the levels of vitamin B12. Risk estimate is done using odds ratio which interpreted that there is a substantial reduction in vitamin-B12 levels in T2DM patients using metformin for 1 -10 years.

Variables	Total	Cases	Controls
No. of Participants	68	34	34
Age	46.10±14.6	53.38 ± 12.11	38.61±13.22
Sex (%) Female	32 (47.1)	16 (47.1)	16 (47.1)
Male	36 (52.9)	18 (52.9)	18 (52.9)
HbA1c %	9.07 ±2.67	9.07 ±2.67	-
Vitamin-B12 level, pg/ml	349 ± 156.3	350.1±175.2	349.6±137.49
Vitamin-B12 deficiency (<200 pg/ml), in (%)	13(19.1)	7 (20.5%)	3 (8.82%)
Haemoglobin g/dl	12.68 ± 2.1	12.17±2.03	13.19±2.21
Haematocrit %	39.36 ± 6.61	38.07±6.18	40.65±6.87
MCV	84.50 ± 6.293	85.62 ± 4.67	83.38±7.48

Table 1. Clinical Characteristics of the Study Population

Vitamin-B12	N/N	Microcytic/ hypochromic	N/N	Microcytic hypochromic HS+	N/H HS+	Dimorphic HS+	Macrocytic HS+	N/H
<200 pg/ml	1	2	0	2	6	2	2	1
200 - 300 pg/ml	0	1	0	0	3	1	0	1
300 - 400 pg/ml	1	1	0	0	1	0	0	1
400 - 500 pg/ml	0	1	1	0	1	0	0	1
>500 pg/ml	2	0	0	0	2	0	0	0
Total	4	5	1	2	13	3	2	4
	34							

Table 2. Vitamin-B12 and Peripheral Smears Cross Tabulation in Cases

Metformin Years of Use	Vitamin-B12					
	< 200 pg/ml	200 – 300 pg/ml	300 – 400 pg/ml	400 – 500 pg/ml	>500 pg/ml	Total
>6 months	0	1	0	2	0	3
1 – 10 years	5	5	7	3	4	24
10 – 20 years	2	1	0	1	1	5
20 – 30 years	0	1	0	0	1	2
Total			**34**			

Table 3. Metformin Years of use and Vitamin-B12 levels Cross Tabulation in Cases

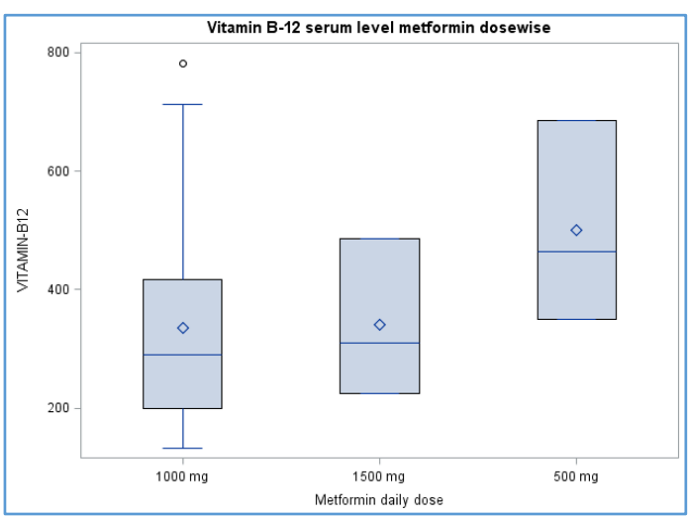

Figure 1. Box and Whisker Plot Depicting Metformin Dosage and Serum Vitamin B12 Levels

Paired Samples Correlations			
		N	Correlation
Pair 1	Age & Vitamin B12	68	-0.013

Table 4a. Paired Samples Correlation

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for VAR00001 (cases / controls)	1.000	0.287	3.480
For cohort Vitamin B12 = 1.00	1.000	0.358	2.792
For cohort Vitamin B12 = 2.00	1.000	0.802	1.246
No. of Valid Cases	68		

Table 4b. Risk Estimate (Odds Ratio)

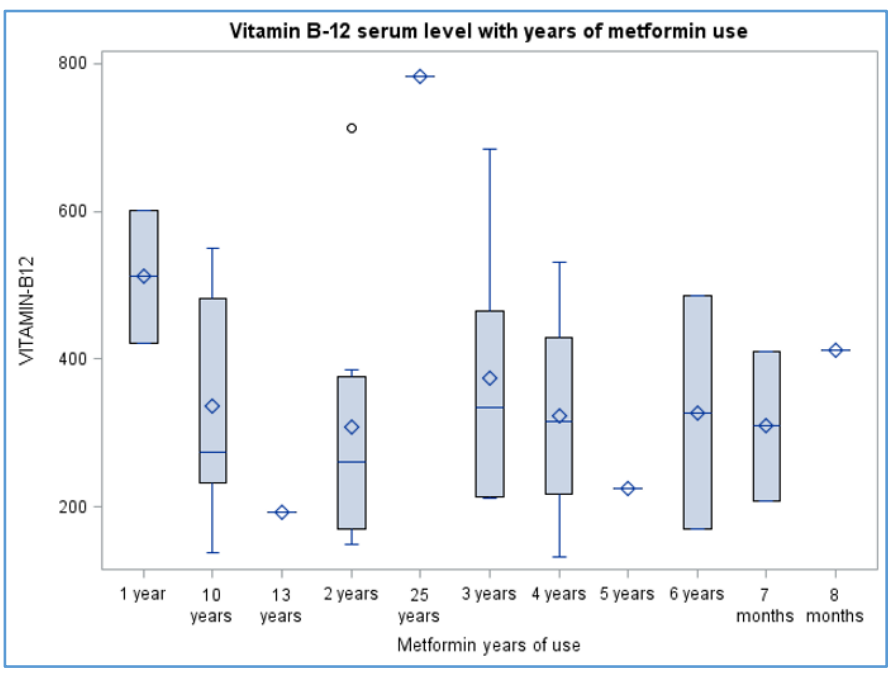

Figure 2. Box and Whisker Plot for Vitamin B12 Levels and Duration of Metformin Use

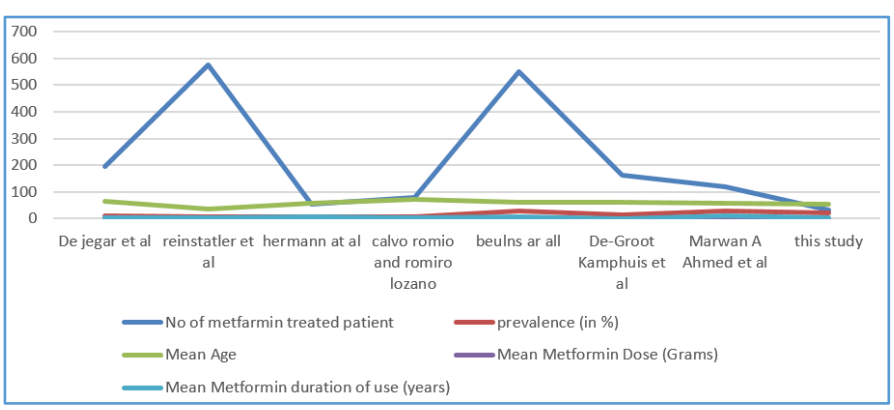

Figure 3. Table 5 Depicted in Line Chart Comparing the Characteristics of 8 Studies

DISCUSSION

In the present study the prevalence of vitamin-B12 deficiency in T2DM patients receiving metformin is high - 20.5% when compared to the age matched non-diabetic study controls - 8.82%. The prevalence of vitamin B12 deficiency as reported Anil Kumar R.et al was 27.33% in T2DM subjects on metformin therapy and the percentage of deficiency increased with increase in dosage of metformin with no correlation to duration of metformin therapy.[6] Our study showed a clear relationship between the dosage or length of metformin use and vitamin B12 deficiency in patients with type 2 diabetes though comparing the obtained prevalence with the results of previous studies is not straightforward and should consider several factors. The prevalence rates of vitamin B12 deficiency (<191 ng/L) were 27% and 12% in Europeans and Indians respectively, and higher in metformin treated type 2 diabetes patients.[7,8,9,10,11]

Table 5 shows the comparison of the present study with with other studies that used similar deficiency cutoff, ranging between 145 – 150 pmol/l.[12,13,14] The table reveals study-related factors with potential to affect the obtained prevalence, including mean participants age, mean metformin daily dose, study settings, mean metformin duration of use and whether participants with renal impairment were excluded. Our reported prevalence is high relative to previously reported estimates.

Study	Number of Metformin-Treated Patients	Prevalence	Mean Age (Years)	Mean Metformin Dose (Gram)	Mean Metformin Duration of Use (Years)	Study Setting	Exclusion of Renally Impaired Patients
De Jager et al.[15]	196	9.9 %	64	2.1	4.3	Outpatient clinics of 3 nonacademic hospitals, The Netherlands	Yes
Reinstatler et al.[16]	575	5.8 %	36.4	NA	5	NHANES sample, United States	Yes
Hermann et al.[17]	53	8 %	58.5	2.2	5.2	Outpatient diabetes clinic of a general hospital, Sweden	Yes
Calvo Romero and Ramiro Lozano[18]	81	8.6 %	71.	1.8	3.6	Internal medicine clinic of a first level hospital, Spain	No
Beulens et al.[19]	550	28.1 %	61.6	1.3	5.3	4 primary care centers, The Netherlands	No
De Groot-Kamphuis et al.[20]	164	14.1 %	62.6	-	4.9	Secondary care outpatient diabetes clinic, The Netherlands	No
Marwan A. Ahmed et al.[21]	121	28.1%	58.5	2.4	9.6	Outpatient diabetes clinics of 2 tertiary hospitals, South Africa	Yes
This Study	34	20.5 %	53.3	1	2.83	Out-patient depart & In-patient wards of RVM Hospital, India	Yes

Table 5. Characteristics of Studies that Measured the Prevalence of Metformin- Induced Vitamin-B12 Deficiency with Diagnostic Cut-Points Ranging between 145-150 pmol/L [21]

The mean HbA1C in our study cases is <9.07 ±2.67 reflecting either poor control of diabetes despite high doses of Metformin or lack of compliance.

The mean serum Vitamin B12 levels in T2DM cases on metformin is 350.1±175.2 and is 349.6±137.49 in non-diabetic controls of this study. Ko S-H, et al. in a Korean study reported that the mean vitamin B12 level in patients with diabetes using metformin was 665.7±246.7 pg/mL.[22]

The mean serum vitamin B12 levels among American adults with diabetes were 430.2±13.0 pg/mL in metformin users and 524.0±10.6 pg/mL in non-metformin users and 475.3 ±3.9 pg/mL in non-diabetic adults.[23]

The observed mean MCV level in our subjects with vitamin B12 deficiency was 85.62 ± 4.67 and the prevalence of megaloblastic anemia was about 11.35% and dimorphic anemia 11.35%.

Megaloblastic anaemia is widely regarded to have an increased MCV (MCV>100 fL) but previous reports have indicated that up to 30% of vitamin B12 responsive disorders have normal MCVs[24] Ko S-H, et al. reported mean MCV as >100fl and prevalence of macrocytic anaemia as 0.5% in their study.

The difference in the mean MCV between the groups with and without vitamin B12 deficiency was not very significant. The mean MCV in the study cases is 85.62 ± 4.67

and in controls is 83.38±7.48. The MCV did not differ between T2DM-met patients with and without B12 deficiency (89.4 versus 89.0; P = 0.153).[25]

Macrocytosis in this study is seen in 22.7% (Macrocytic –11.35% and Dimorphic – 11.35%) of the B12 deficient group when compared to 9.6% (9.6% versus 0.6%; P <0.001) reported by Charbel Pereira Damiao et al.

Careful examination of the peripheral blood smear could be helpful in distinguishing vitamin B12 deficiency-related anaemia from anaemia of other causes[26]

Hyper-segmented polymorphs were reported in 50% of cases in our study. A high MCV with oval macrocytes and hypersegmented neutrophils suggests folate or cobalamin deficiency and is an indication for assays of these vitamins.[27]

When an anaemic patient presents with Hb <10 g/dl and MCV in the range of 80–99 fl, measurement of vitamin B12/folate should be considered if both the RDW is ≥16% and the reticulocyte index is ≤2%.

Table 3 cross tabulates vitamin-B12 levels with metformin years of use in T2DM patients in which the prevalence of vitamin-B12 deficiency was found within 1- 10 years use of metformin. Therefore, annual screening of serum vitamin B12, serum methylmalonic acid (MMA)/serum homocysteine level should be done in cases taking metformin for more than four to five years with average dose of >1g per day, even in the absence of haematological or neurological abnormalities.[28,29]

In table 4, paired samples correlation and Risk estimate is done showing that an increase in age decreases the levels of vitamin B12 and risk estimate interprets that there is substantial reduction of vitamin-B12 levels in T2DM patients using metformin for 2.83 years(mean).[30,31,]

Limitations

This study was conducted in a tertiary care hospital, raising the possibility of over-representation of patients with complicated T2DM, higher doses and durations of use of metformin.

The study has only measured serum vitamin B12 levels to assess the vitamin status and did not measure homocysteine levels. Reduction in vitamin B12 increases the plasma concentrations of Homocysteine and the current recommendations suggest adding methylmalonic acid or homocysteine tests for better assessment of the intracellular status of the vitamin.[32]

The absence of data regarding compliance to metformin is also a limitation in our study. Compliance can have an impact on both the response to metformin and the levels of vitamin B12.

Hematologic parameter - reticulocyte count has not been taken into consideration in this study.

A much larger study sample is needed to conclusively answer the secondary aim of investigating the relationship between metformin years of use and vitamin B12 deficiency and to show a clinically important difference as small as 10 % to be statistically significant.

CONCLUSION

This prospective case-control study conducted in our institute in rural Telangana region confirms that long-term treatment with metformin in patients with T2DM is associated with higher chances of developing vitamin B12 deficiency, which correlated with hematologic parameters when compared to non-diabetic age matched controls. This study though limited by the sample size, demonstrated that the prevalence of vitamin B12 deficiency, defined by levels <200 pg/ml, in metformin treated T2DM patients was as high as 20.5%. There is a definite need for further research in this regard considering the present study as a pivotal study. Periodic complete blood picture/peripheral smear study for detection of hyper-segmented polymorphs/macrocytes followed by biochemical analysis where needed can be cost effective in rural areas.

ACKNOWLEDGEMENT

Our sincere thanks to Dr. Bheeshma, Head of Department of Pathology– RVMIMS & RC and Dr. Shashidhar Reddy, Assistant Professor of General Medicine – RVMIMS & RC.

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