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Original Research Article

The vitamin B12 levels in type 2 diabetes mellitus patients on metformin and not on metformin

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is one of the major global public health concerns, metformin is one of the most widely used drugs and considered as first-line therapy for management of T2DM. Vitamin B12 malabsorption is observed in patients on metformin therapy leading to biochemical and clinical vitamin B12 deficiency. The aim of the current study was to assess the relationship between metformin therapy and development of vitamin B12 deficiency.
Methods: Current observational cross sectional study was conducted at Madras medical college and Rajiv Gandhi Government general hospital. Serum vitamin B12 and other blood investigation parameters of T2DM patients, on metformin therapy for long duration (6 months or more than 2 years) were measured and correlated with vitamin B12 levels of T2DM patients not on metformin therapy. Patients were given appropriate treatment and were regularly followed up.
Results: Results of the current study findings depicted that significant difference was observed in percent haemoglobin, total blood cells, platelet count, mean corpuscular volume (MCV) and albumin values of patients on metformin therapy when compared to patients who were not on metformin therapy. Study findings also revealed that substantial difference in vitamin B12 deficiency was observed in T2DM patients based on the duration of metformin therapy.
Conclusions: Current study revealed that, metformin therapy for 2 years or more, can lead to significant vitamin B12 deficiency which is also associated with macrocytosis. It was concluded that longer the duration of metformin therapy, more significant would be vitamin B12 deficiency.

Keywords: Type 2 diabetes mellitus, Metformin, Vitamin B12 deficiency

INTRODUCTION

Diabetes mellitus type 2 (T2DM) is the most common type of diabetes which occurs when the body becomes resistant to insulin or doesn't make enough insulin leading to elevated blood glucose levels, which eventually over the period of time damages vital organs of human body like heart, blood vessels, eyes, kidneys and nerves.[1,2] Diabetes mellitus (DM) is one of the major global public health concerns; the prevalence of type 2 diabetes has increased substantially in past three decades. International diabetes federation has estimated that in 2017, 451 million of adult population worldwide lives with diabetes with a projected increase to 693 million by 2045. Diabetes is one of the major causes of mortality. Together with cancer, cardiovascular, and respiratory disease, diabetes account for over 80% of deaths caused due to non-communicable diseases (NCDs).[3-5]

Metformin, is a guanidine derivative, extracted from the plant *Galega officinalis* and primarily used for the treatment of type 2 diabetes mellitus for more than 60 years.[6] Metformin is one of the most widely used drugs in the treatment of T2DM since its approval in the United Kingdom in 1958 and in the United States in 1995, with doses ranging from 500 to 2,500 mg/day.[7] It is the first-line therapy for patients with T2DM according to the American diabetes association/European association for

study of diabetes guidelines. Metformin reduces serum glucose level mainly by nonpancreatic mechanism.[8] Metformin does not increase the insulin secretion but increases the effects of insulin.[9]

In addition metformin also suppresses the endogenous glucose production by reducing the rate of gluconeogenesis and glycogenolysis in liver. Moreover, metformin activates the enzyme adenosine monophosphate kinase (AMPK) resulting in the inhibition of key enzymes involved in gluconeogenesis and glycogen synthesis in the liver.[8-11] Common side effects of metformin include gastrointestinal symptoms (incidence rate 20-30%), like nausea and vomiting, and lactic acidosis (incidence rate 1/30,000), mainly in diabetic patients with liver and kidney dysfunction.[12]

Vitamin B12 (cobalamine) is an essential water soluble micronutrient required for optimal hemopoetic, neuro-cognitive and cardiovascular function.[13] Vitamin B12 plays a vital role in DNA synthesis.[14] Published literature and case reports have documented biochemical and clinical vitamin B12 deficiency to be highly prevalent among patients with type 1 and type 2 diabetes mellitus.[15-17] Metformin use has been unequivocally demonstrated as the prime factor associated with vitamin B12 deficiency among patients with T2DM. Cross sectional, retrospective, and longitudinal observational studies revealed evidence of vitamin B12 malabsorption in patients who had been treated with metformin for 3 months or in patients on long-term metformin therapy.[18-20] Prevalence of metformin induced vitamin B12 deficiency are reported to be in the range of 5.8% to 33%. The risk of developing metformin associated vitamin B12 deficiency is greatly influenced by increasing age, metformin dose and duration of use as per published reports.[21] Decrease in vitamin B12 absorption due to metformin use can be observed as early as in the fourth month and clinically overt features manifest by 5-10 years.[22] Proposed mechanisms to explain metformin induced vitamin B12 deficiency among T2DM patients include: alterations in small bowel motility, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels, interaction with the cubulin endocytic receptor and inhibition of calcium dependent absorption of vitamin B12-IF complex at the terminal ileum.[23-25]

Aim and objectives

Due to the diverse definitions of vitamin B12 deficiency used in most studies and the cultural and religious beliefs in different regions of the world, comparison of the prevalence of vitamin B12 deficiency among T2DM patients and healthy general populations is difficult. Thus current study was designed with the aim to assess the relationship between metformin therapy and development of vitamin B12 deficiency. The objective of the present study was to determine vitamin B12 levels in T2DM patients on metformin therapy and to correlate it with vitamin B12 levels in T2DM patients not on metformin therapy.

METHODS

Study design, place and duration

Current study was an observational cross section study carried out at institute of internal medicine, institute of diabetology, Madras medical college and at Rajiv Gandhi Government general hospital, Chennai, for the period of one year from April 2018 to March 2019.

Study population and sample size

110 type 2 diabetes mellitus patients, on or not on metformin therapy who attended the diabetology and medicine outpatient department of Madras medical college and Rajiv Gandhi government general hospital during the study period were involved in the study depending on metformin dose and therapy duration.

Inclusion criteria

Inclusion criterion for the patients to be enrolled in current study were; T2DM patients in the age group of 18 to 80 years, who were on metformin therapy or not on metformin therapy were included depending on dose and therapy duration, all T2DM patients who were on metformin therapy for more than 6 months or 2 years and all the patients who gave their consent to participate were included in the study.

Exclusion criteria

Patients with irregular treatment, anemia, pancytopenia, gastritis, immune disorders, patients having vegetarian diet, chronic ill-nourished patients, chronic alcoholics or lactating mothers, patients of age group less than 18 or greater than 80 years and patients not willing to participate were excluded from the study.

Procedure

A detailed case history of each patient with reference to name, age, sex, address, contact number, outpatient number, occupation, presenting complaints with duration, treatment history, associated comorbid illness, history of any drug intake for other conditions, any similar complaints in the family members, was documented and recorded.

General and systemic investigations and vitals were done and recorded. Blood investigations such as complete blood count, fasting blood sugar (FBS), post prandial blood sugar (PPBS), vitamin B12 were performed after obtaining proper informed written consent from the patients. Patients participating in the study were given appropriate treatment and were regularly followed up.

RESULTS

Results of distribution study of total 110 patients based on parameters like age group, sex, duration of therapy and peripheral smear study are depicted in (Table 1), distribution studies were performed after broadly categorizing participating patients in two groups; patients on metformin therapy and patients not on metformin therapy. Results of peripheral smear study indicated that about 65% patient on metformin therapy exhibited macrocytosis and about 74% of patients not on metformin therapy were normal.

Results of the current study findings depicted that the mean haemoglobin value, total blood cells and platelet count were observed to be significantly decreased in patients on metformin therapy. It was observed based from the distribution study that the mean corpuscular volume (MCV) was significantly higher in patients on metformin therapy. The mean corpuscular hemoglobin (MCH), lactate dehydrogenase, fasting blood sugar (FBS), post prandial blood sugar (PPBS), uric acid, total bilirubin (TB), mean direct bilirubin (DB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase and creatinine values were observed to be almost similar in both the groups with no significant difference. In current investigation significant difference was observed in mean albumin and mean vitamin B12 values in the patients on metformin therapy when compared to patients who were not on metformin therapy. The current study findings revealed that substantial difference in vitamin B12 deficiency was observed based on the duration of metformin therapy (Table 3).

Table 1: Distribution study of patients based on parameters like age group, sex duration of therapy and peripheral smear study.

Parameter	Metformin therapy				Total		Pearson Chi square coefficient	P value
	No		Yes		N	%		
	N	%	N	%				
Age group (years)								
31-40	10	13.2	1	2.9	11	10.09	8.057	0.089
41-50	25	32.9	10	29.4	35	31.89		
51-60	20	26.39	16	47.19	36	32.7		
61-70	8	10.59	5	14.79	13	11.8		
Above 70	13	17.19	2	5.99	15	13.6		
Total	76	100	34	100	110	100		
Sex								
Male	51	67.1	24	70.6	75	68.2	0.1	0.717
Female	25	32.9	10	29.4	35	31.8		
Total	76	100	34	100	110	100		
Duration of therapy								
<6 months	10	13.2	10	29.4	20	18.2	9.687	0.001
>6 months	66	88.6	24	70.6	90	81.89		
Total	76	100	34	100	110	100		
>2 years	68	89.5	22	64.7	90	81.8		
<2 years	8	10.5	12	35.3	20	18.29		
Total	76	100	34	100	110	100		
Peripheral smear study								
Macrocytosis	0	0	22	64.7	22	20	65.315	0.001
Microcytic hypochromic	10	13.2	1	2.9	11	10		
Microcytic normochromic	9	11.8	5	14.75	14	12.79		
Normal	56	73.7	6	17.69	62	56.49		
Normocytic normochromic	1	1.3	0	0	1	0.99		
Total	76	100	34	100	110	100		

Table 2: Distribution study of patients based on blood investigation parameters.

Parameter	Metformin therapy	N	Mean	SD	Standard error mean	t value	P value
Hemoglobin (gm/dl)	No	76	11.4	1.48	0.1707	6.603	0.001
	Yes	34	9.4	1.49	0.2568		
Total count	No	76	7373	2337	268	4.704	0.001
	Yes	34	4957	2802	480		

Continued.

Parameter	Metformin therapy	N	Mean	SD	Standard error mean	t value	P value
MCV	No	76	80.67	2.4144	0.2769	11.114	0.001
	Yes	34	104.74	18.6395	3.1966		
MCH	No	76	30.894	1.519	0.1742	0.881	0.381
	Yes	34	30.588	2.016	0.3458		
Platelet count	No	76	3.01	0.69	0.079	7.490	0.001
	Yes	34	1.71	1.12	0.19		
LDH	No	76	252.40	42.24	4.846	1.043	0.299
	Yes	34	242.85	48.96	8.396		
FBS	No	76	135.23	6.72	0.7716	-l .410	0.161
	Yes	34	137.11	5.82	0.9997		
PPBS	No	76	238.57	15.93	1.827	-0.068	0.946
	Yes	34	238.79	13.94	2.392		
Uric acid	No	76	5.250	0.9823	0.1134	-0.457	0.649
	Yes	34	5.338	0.7901	0.1355		
TB	No	76	0.9039	0.1872	0.0214	-0.122	0.903
	Yes	34	0.9088	0.2065	0.0354		
DB	No	76	0.6684	0.7492	0.0859	0.480	0.633
	Yes	34	0.6059	0.1739	0.0294		
SGOT	No	76	25.447	5.402	0.6196	-l.872	0.064
	Yes	34	27.647	6.314	1.082		
SGPT	No	76	22.118	4.901	0.5622	0.440	0.661
	Yes	34	21.676	4.790	0.8216		
Albumin	No	76	3.976	0.268	0.030	9.148	0.001
	Yes	34	3.288	0.520	0.089		
ALP	No	76	72.644	17.443	2.009	-0.710	0.479
	Yes	34	75.088	14.810	2.54001		
Vitamin B12	No	76	455.828	69.90	8.018	19.225	0.001
	Yes	34	198.441	51.71	8.868		
Creatinine	No	76	0.8276	0.14104	0.01618	2.144	0.034
	Yes	34	0.7676	0.12240	0.02099		

Table 3: Vitamin B12 deficiency study based on duration of metformin therapy.

Duration of metformin therapy	Vitamin B12 values			
	Mean	Standard deviation	Minimum	Maximum
More than 6 months	490.50	61.92	358.00	567.00
More than 2 years	350.89	134.88	117.00	567.00

DISCUSSION

Metformin is the commonly used drug for the treatment of diabetic mellitus.[7] It is reported and observed that metformin interrupts vitamin B12 absorption and cause vitamin B12 deficiency.[25]

In current observational cross sectional study, serum vitamin B12 and other blood parameters of T2DM patients who were on metformin therapy for long duration (6 months or more than 2 years) were measured and compared with vitamin B12 levels of T2DM patients who were not on metformin therapy. The observed results were correlated with various parameters like FBS, PPBS, MCV, MCH, total counts, hemoglobin, platelet count, etc.

In current study, out of 110 patients, 75 were males and 35 females. Out of 75 males, 51 were on metformin and 24 were not on metformin therapy and out of 35 females 25 on were on metformin therapy and 10 were not on metformin therapy. 71% of total enrolled patients were on metformin therapy from 6 months or more and 29% were on metformin therapy from less than 6 months, whereas 65% of patients were on metformin therapy from more than 2 years and 35% were on metformin therapy from less than two years. Age and sex wise distribution studies of patients revealed no significant difference. Significant difference was observed in % hemoglobin, total blood cells count, platelet count, MCV value and vitamin B12 values between the diabetes mellitus patients who were on metformin therapy, when compared to patients not on metformin therapy. Totally 34 patients who were on metformin therapy for more than 2 years were observed to

have an increased MCV value. In current study findings significant correlation was observed between metformin based therapy for long duration and vitamin B12 deficiency. Statistical studies of current study findings did not indicate and significant correlation between the parameters like sex, age and vitamin B12 deficiency. Current study findings revealed that there was a significant correlation between vitamin B12 deficiency and metformin therapy for 2 years whereas the correlation was observed to be non-significant when the duration of metformin therapy was for 6 months thus it was concluded that more the mean duration of metformin therapy, more were the chances for development of vitamin B12 deficiency.

No significant correlation could be established from current study findings in parameters like MCH, LDH, FBS, PPBS, uric acid, TB, DB, SGOT, SGPT, ALP and creatinine as the observed values of these parameters were almost similar for patients on metformin therapy and for patients not on metformin therapy. Peripheral smear results revealed a significant difference in some parameters between the patients who were on metformin therapy and patients who were not on metformin therapy. Among the patients who were on metformin therapy, 65% had macrocytosis, 15% were microcytic normochromic, 18% were normal and 3% were microcytic hypochromic. Among the patients who were not on metformin therapy 74% were normal, 13% were microcytic hypochromic, 12% were microcytic normochromic and 1% were normocytic normochromic.

The National health and nutritional survey done in US from 1999-2006 had documented vitamin B12 deficiency more among patients on metformin therapy.[21] Similar to current study findings, Pflipsen et al and Shobha et al reported high prevalence of vitamin B12 deficiency among patients of diabetes mellitus who were on metformin therapy.[26,27] Aroda et al reported that duration of metformin therapy is a main factor leading to vitamin B12 deficiency, which is similar to current study findings.[20]

Limitations

Limitations of the current study were: the sample size of the investigated study group was small due to time and financial constraints and more concrete results and recommendations could have been made with a larger sample size. Dose of metformin was not calculated in current study, so the relationship between total cumulative dose and vitamin B12 deficiency could not be established.

CONCLUSION

It can be concluded form current study findings that, metformin therapy for 2 years or more, can lead to significant vitamin B12 deficiency which is also associated with macrocytosis. It was concluded that longer the duration of metformin therapy, more significant is vitamin B12 deficiency. No significant correlation was observed and concluded for between metformin therapy and vitamin B12 deficiency for patients who were not on metformin therapy or were on therapy for less than 6 months. It was also concluded that serum vitamin B12 assay helps to assess early vitamin B12 deficiency in patients who are on metformin therapy.

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