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A cross sectional study to estimate the prevalence of vitamin B12 deficiency among type 2 diabetes cases on metformin therapy: a one year study

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ABSTRACT

Background: Type 2 diabetes mellitus is associated with long term neurological complications. Metformin usage as first line drug for longer duration is associated with Vitamin B12 deficiency. Metformin on longer usage is associated with B12 deficiency in various studies and prevalence is variable. The study aims to estimate the prevalence of Vitamin B12 deficiency and its associated risk factors among T2DM on metformin therapy.

Methods: A cross sectional study for one year was conducted at Narayana Medical college a tertiary care hospital. All T2DM cases attending the department and on Metformin usage were enrolled and data of age, sex, duration of T2DM, dosage of Metformin were noted as variables. Levels of Hb1Ac and complete blood picture were noted. Statistical analysis was conducted by Statistical package for social sciences (SPSS) version 21 v and p<0.05 was considered statistically significant.

Results: A total of 258 participants were enrolled of which 68 (26.36%) were B12 deficient, 46 cases (17.83%) moderately deficient and 144 (55.81%) were normal. Prevalence of Vitamin B12 in the study was 26.36%. There was a statistically significant association between Vitamin B12 levels and daily dose of metformin usage. (p>0.005)

Conclusions: Our study clearly highlighted the association of Vitamin B12 deficiency among T2DM on metformin therapy. Prevalence of Vitamin B12 deficiency among T2DM patients on metformin is quite considerable and cannot be neglected. Physicians prescribing the drug should plan inclusion Vitamin B12 supplementation as regular drug in T2DM cases.

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

INTRODUCTION

Diabetes mellitus is a global health problem with an estimated prevalence of 8.55 among the adult population with a gradual increase in the decade. Raising prevalence of DM is observed mostly in low and middle-income countries with Asian continent becoming the epic center for diabetes. Type 2 diabetes mellitus(T2DM) is the most common type of DM. India ranks second next to China with around 69.2 million cases of T2DM. Due to chronic

nature of the disease, T2DM is associated with long term complications predominantly affecting the nervous system and other major systems of the body. Hence to overcome the long-term complication, metformin is used as the first line option in management and treatment of diabetes globally by all the associations. This first line recommendation is based primarily on metformin's glucose-lowering effects, relatively low cost, and generally low level of side effects, including the absence of weight gain.^{2,3} Metformin has been shown to reduce

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insulin dose requirement, HbA1c (0.6–0.9%), weight, and total cholesterol. Metformin provided greater protection against the development of macrovascular complications than would be expected from its effects upon glycaemic control alone.

However, in cases of T2DM with long-term usage of metformin, literature from the studies have documented association of Vitamin B12 deficiency in addition to the beneficial effects. This association of Vitamin B12 deficiency is due to calcium-dependent ileal membrane antagonism, an effect which can be antagonised with supplemental calcium. This deficiency is associated with dose and duration of metformin usage and is reported to occur among patients that use it for more than three years and in higher doses.4 India being a country with diverse racial population, eating habits, cultural traditions and associated increase in the incidence of T2DM this duration and dosage of metformin among the cases is variable. Hence a study to associate the dose and duration of metformin use among T2DM with Vitamin B12 is required in Indian population. Early detection of Vitamin B12 deficiency is important to reduce further neurological and haematological complication.⁵ Hence a regular screening and periodical monitoring of Vitamin B12 levels among the T2DM on metformin usage may be a considered useful tool in diabetes.

The present study was first of its kind in south India to assess the prevalence of Vitamin B12 deficiency among type 2 Diabetics who are on metformin therapy and associated factors in development of deficiency.

METHODS

The present prospective cross-sectional study was conducted for a period of one year from January 2020 to December 2020 at a tertiary care hospital of south India. The study was conducted by the department of General medicine. The study protocol was presented before the Institutional ethical committee and was approved. The study was conducted as per the guidelines of the committee and was reviewed at the end of the study. The study details were clearly explained to the participants in the study in regional language and a written informed consent was obtained from the participants who consented to participate in the study.

All the patients attending the diabetic clinic of the general medicine department were thoroughly screened for the inclusion criteria and the study details were explained and those who fulfilled the eligible criteria and consented were included in the study. Inclusion criteria for the study participation were diagnosed cases of Type 2 diabetes mellitus with minimum duration of 12 months and on metformin therapy for a minimum period of 12 months. Patients who were not willing to participate, on insulin therapy, on proto pump inhibitors, receiving Vitamin B12 therapy, pure vegetarians, HIV positive, diagnosed cases

of pernicious anemia, malabsorption syndromes were excluded from the study.

Demographic details of the study participants including age, sex and study variables like duration of T2DM, duration of metformin therapy, dosage of metformin regularly, smoking and alcoholism. All the participants of the study were subjected to thorough clinical examination by history taking, signs and symptoms of Vitamin B12 (Peripheral neuropathy). Deficiency Laboratory investigations included a complete blood picture (CBP), Vitamin B12 estimation and HbA1C (Glycated hemoglobin). 5 ml of venous blood sample was collected and CBP was analyzed by using Beckman Coulter, USA. Anemia was defined as blood haemoglobin level of <13 g/dl in males and, 12 g/dl in females as per WHO guidelines.⁶ Hb1Ac was assayed by using COBAS INTEGRA 400 plus Roche, Switzerland which estimates Hb1Ac by turbidimetric inhibition immunoassay. Vitamin B12 levels in the serum were quantified by using a chemiluminescent enzyme immunoassay (Beckman Coulter, Inc. USA). The participants were classified into three categories based on their serum vitamin B12 measurements: a deficient group (<133 pmol/L), a borderline deficient group (133-200 pmol/L) and a normal group (200 pmol/L).7

Statistical analysis

Statistical package for social sciences (SPSS) version 21 Newyork, USA was used for analysis of the collected data. Mean and standard deviation was calculated for all the variables, categorical variables were analysed using Chi-Square test. A p<0.05 was considered statistically significant.

RESULTS

A total of 258 cases who consented for the study and fulfilled the inclusion criteria were enrolled as study participants for a period of one year. Female participants dominated the study with 53.49% while males with only 46.51%. Majority of the cases were in age group of 51-60 years (34.11%) followed in order by >60 years (24.42%), 41-50 years (24.03%) and the least in age group of 30-40 years (17.44%). The mean age of the cases in the study was 55.21±4.89 years. The mean duration of T2DM in the study was 12.54±4.38 years. Majority of the participants 91 (35.27%) were with T2DM duration >10 years and 34.50% between >5 - <10 years and only 30.23% with <5 years duration. The mean dosage of metformin usage in the study was 1678±148 mg and the mean duration of use in the study was 12.41±4.6 years. (Table 1)

In the present study, 28.68% of cases had HbA1C% >6.5% with poor glycemic control, 24.81% with borderline and 46.51% with good glycemic control. The mean HbA1c value in the study was $6.2\pm1.1\%$. (Figure 1)

In the present study, of the 258 cases 68 (26.36%) were Vitamin B12 deficient (<133 pmol/L) and 46 (17.83%) were borderline deficient (133-200 pmol/L) and 144 (55.81%) were with normal Vitamin B12 levels (200 pmol/L). The mean serum concentration of Vitamin B12 was 260.12 pmol/L. The prevalence of Vitamin B12 deficiency in the present study was observed to be 26.36%. (Figure 2)

Table 1: Demographic features and other characteristics of cases in the study (n=258).

Total cases	N				
Male	120	46.51			
Female	138	53.49			
Age group (in years)					
31-40	45	17.44			
41-50	62	24.03			
51-60	88	34.11			
>60	63	24.42			
Mean age of cases	55.21	± 4.89			
Duration of T2DM (in years)					
<5	78	30.23			
>5 -<10	89	34.50			
>10	91	35.27			
Mean duration	12.54	12.54±4.38			
Dose of metformin (in mg)					
<2000	108	41.86			
≥2000	150	58.14			
Mean dose	1678±	-148 mg			
Duration of metformin use (in years)					
<4	48	18.60			
> 4-<10	94	36.43			
>10	116	44.96			
Mean duration (years)	12.41:	±4.6 years			

In the present study, 84 cases (32.56%) were found to be anemic, of which 20 participants were Vitamin B12 deficient (23.81%), 32 (38.10%) were borderline deficient and other 32 were normal (38.10%). There was no statistically significant association between anemia and Vitamin B12 deficiency in our study. Peripheral neuropathy was observed in 44 cases (52.38%) of which 16 cases were with Vitamin B12 deficient and 28 cases were identified with borderline deficiency of Vitamin B12. (Table 2) There was no statistically significant association between peripheral neuropathy and Vit B12 deficiency. (p>0.005)

Table 3 clearly explains the association of characteristics and laboratory findings of the cases with regard to distribution of Vitamin b12 serum concentrations as deficient, borderline and normal categories. No significant associations were found between serum vitamin B12 levels and age, gender, duration of DM and haemoglobin

and HbA1c levels. There was a statistically significant association between Vitamin B12 levels and daily dose of metformin usage. (p<0.005)

Table 2: Tabulation of vitamin B12 status and peripheral neuropathy.

Peripheral Neuropathy					
Vitamin B12 status	Absent	Present	Total		
Normal	144 (100%)	0	144		
Borderline deficiency	18 (39.13%)	28 (60.87%)	46		
Deficiency	52 (76.47%)	16 (23.53%)	68		
Total	214 (82.95%)	44 (17.05%)	258		

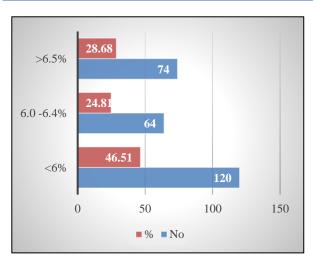


Figure 1: Levels of Glycosylated haemoglobin among the cases in the study.

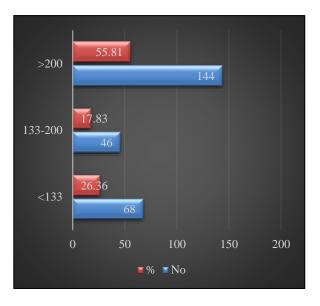


Figure 2: Vitamin B12 levels and categories of deficiency among the cases in the study.

Table 3: Characteristics and laboratory findings of cases in the study as per vitamin B12 levels (n=258).

Characteristics and	Vitamin B12 category			_ Total	P value	
laboratory finding	Deficient	Borderline	Normal	Total	1 value	
Total	68 (26.36%)	46 (17.83%)	144 (55.81%)	258 (100%)		
Age in years ± SD	54.12±3.54	53.28±5.14	51.48±2.98	55.21±4.89	>0.005	
Gender						
Male	38 (31.67%)	24 (20%)	58 (48.33%)	120 (46.51%)	>0.005	
Female	30 (21.74%)	22 (15.94%)	86 (62.32%)	138 (53.49%)	>0.005	
Mean duration of DM duration in years	12.98±2.46	11.84±8.64	12.87±6.84	12.54±4.38	>0.005	
Daily dose of Metformin in mg.						
<2000	24 (22.22%)	12 (11.11%)	84 (77.78%)	108 (41.86%)	<0.005	
≥ 2000	44 (29.33%)	34 (22.67%)	60 (40%)	150 (58.14%)		
Mean daily metformin dose in $mg \pm SD$	1948±326 mg	1854±184 mg	1684±142 mg	1678±148 mg	< 0.005	
Duration of Metformin use (in years)						
<4	14 (20.59%)	12 (26.09%)	22 (19.3%)	48 (18.6%)	>0.005	
> 4-<10	22 (32.35%)	10 (21.74%)	62 (43.06%)	94 (36.43%)		
>10	32 (47.06%)	24 (52.17%)	60 (41.67%)	116 (44.96%)		
Mean laboratory findings ± SD						
Hb1Ac	6.4±8.1	6.8±7.1	6.8±4.7	6.2±1.1	>0.005	
Hb in g/dl	11.5 ± 2.4	14.1±5.4	12.9 ± 8.5	12.5 ± 3.2	>0.005	

DISCUSSION

Diabetes is a chronic disease-causing severe life-threatening sequela in its course of illness. Metformin the commonly used 1st line management option is associated with potential benefits when compared to the long-term effects of causing Vitamin B12 deficiency as mentioned in various literature studies. The B12-intrinsic factor complex uptake by ileal cell membrane receptors is known to be calcium-dependent, and metformin affects calcium dependent membrane action, resulting in B12 deficiency. However, the prevalence of Bit B12 deficiency associated with long term metformin usage in T2DM is quite variable and influenced by many factors. Hence the present study was aimed to estimate the prevalence of Vitamin B12 deficiency among T2DM cases.

In the present study the prevalence of Vitamin B12 deficiency was estimated to be 26.36% which is quite similar and also quite variable with some of the studies abroad. Prevalence rate of our study was almost similar to the findings in the study of Marar et al a study from Turkish population who analysed the rates among 400 study population but the mean duration of diabetes and metformin usage was considerably higher than our study. They evaluated association of metformin use and B12 deficiency with anaemia, neuropathy.8 A study by Pflipson et al, on diabetic population has revealed B12 deficiency in range of 22 percent but the study population was evaluated using past medical records, survey for use of insulin, other hypoglycemic agents. In their study, the range of Vitamin B12 deficiency for evaluation was less than 100pg/ml and hence the results are variable from our study population.⁹ A study in an elderly population who lived in long-term institutions reported a prevalence of 53.2%.¹⁰

A similar study performed in Brazil in 2010 showed low B12 levels (<130 pg/ml) in 26.9% and possibly low (125-250 pg/ml) in 36.8% of patients, where the results were comparable to our study.

In our study the association of peripheral neuropathy among the cases with Vitamin B12 deficiency was 52.38% which is quite higher than the studies mentioned earlier. Findings of our study were similar to the reports of Ahmed et al who reported 51% of incidence of peripheral neuropathy among T2DM cases on metformin usage. But the duration of T2DM and dosage of metformin was higher in his study when compared with ours. 11 In our study we concluded that increased metformin dose was associated with Vitamin B12 deficiency with statistical significance (p>0.005). Our conclusion was almost similar with few studies conducted earlier globally. 12 In contrast to previous studies, we found a significant correlation between Hb% among patients on metformin having B12 deficiency as compared to those who are not deficient. This can be explained by the fact that the variables considered in the present study, age factor, duration of T2DM, may be interfering with the correlation between Hb% and Vitamin B12 status. Aroda et al reported a high prevalence of anaemia in the metformin group but no association with vitamin B12 status.13

Limitation of our study was we couldn't measure the levels of methylmelanoic acid which can improve the sensitivity of results. We didn't evaluate folate deficiency and megaloblastic anaemia. There was no control group to compare the results and associate the causal relationship.

CONCLUSION

Our study clearly highlighted the association of Vitamin B12 deficiency among T2DM on metformin therapy. Prevalence of Vitamin B12 deficiency among T2DM patients on metformin is quite considerable and cannot be neglected. Physicians prescribing the drug should plan inclusion Vitamin B12 supplementation as regular drug in T2DM cases. Further studies are required to evaluate effect of B12 replacement in these patients towards reducing B12 deficiency and associated symptoms.

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Ethical approval: The study was approved by the

Institutional Ethics Committee

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