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Vitamin B_{12} Deficiency in Metformin Treated type 2 Diabetic Patients and Factors Associated with it: A Cross-Sectional Study

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Abstract
Objectives: *The objectives of the present study were to assess frequency of vitamin B_{12} deficiency in metformin treated diabetic patients and to evaluate various factors associated with B_{12} deficiency.*
Materials and Methods: *This cross-sectional study involved 82 adult patients of type 2 diabetes treated with metformin for at least one year in a tertiary care government hospital. Absolute vitamin B_{12} deficiency was defined as $\leq$ 203 pg/ml, values of 203 -298 pg/ml were considered as borderline deficiency and values above >298 pg/ml as normal.*
Results: *The mean age of study population was 60.23 ± 9.20 years. The mean daily dose of metformin was 984.7 ± 185.7 mg. About 41% patients received combination therapy with metformin and glimepiride. Multivitamin supplements were used by 37.8% patients. Absolute vitamin B_{12} deficiency was found in 7.31% and combined absolute plus borderline deficiency was found 34.1% patients. Age, gender, duration of diabetes, duration of metformin therapy, total daily dose of metformin, HbA1c value, use of sulfonylurea and use of H_2 antihistaminics was not significantly associated with vitamin B_{12} deficiency. Use of multivitamin led to significant decrease in frequency of vitamin B_{12} deficiency (16.1% vs. 45.1%, p=0.007).*
Conclusion: *Substantial proportion of patients receiving long term metformin show biochemical deficiency of Vitamin B_{12}.Use of multivitamin significantly reduces likelihood of vitamin B_{12} deficiency.*
Keywords: *metformin, vitamin B_{12}, risk factors.*

Introduction

Type 2 diabetes mellitus (T2DM) is a major health concern in India. Metformin a biguanide, that has been shown to reduce micro-vascular and macro-vascular complications of T2DM is recommended as an initial pharmacotherapy by current treatment guidelines.[1] Consequently, metformin is commonly used oral medication in T2DM.

One of the potential adverse effects with long term metformin therapy is vitamin B_{12} deficiency secondary to reduced absorption from its absorption site in terminal ileum.[2] Malabsorption of vitamin B_{12} in 30% of patients receiving metformin on continuous basis was shown in 1971.[3] Afterwards, first clinical trial of efficacy of metformin by DeFronzo *et al.*, and subsequent long term clinical trials, observational studies and meta-analyses concluded that metformin treatment leads to reduced serum vitamin B_{12} levels in a dose dependent manner.[4-8]

Although metformin induced vitamin B_{12} is generally not associated with overt clinical manifestations, it has been linked to worsened peripheral neuropathy, depression and cognitive impairment in T2DM patients.[9-11]Early detection and prompt treatment of vitamin B_{12} deficiency is crucial to prevent irreversible neurological consequences. But unfortunately routine monitoring of serum B_{12} levels in metformin treated T2DM patients is not practiced uniformly.[12]Diabetic patients seeking treatment at government hospitals mainly belong to poor socio-economic strata and are even more vulnerable to vitamin B_{12} deficiency compared to developed countries. [13]

Based on this background information, we hypothesized that higher proportion of metformin treated T2DM patients in our setting would be suffering from vitamin B_{12} deficiency. Determining factors associated with vitamin B_{12} deficiency was also deemed essential. The findings of such study would help to determine the need for vitamin B_{12} screening and/or prophylactic treatment with vitamin B_{12} in vulnerable patients.

Materials and Methods
Ethics Statement
The present study was approved by Institutional Ethics Committee. All participants gave their written informed consent.

Study design and participants
This cross-sectional study was conducted in outpatient setting of a tertiary care government teaching hospital. Consecutive sampling was used to enroll patients during November 2015 to October 2016. Male and female patients of at least 18 years of age diagnosed with T2DM and receiving metformin for more than one year were included in the study. Patients with Type1 DM, pregnant patients, patients suffering from liver, kidney, thyroid disorders, HIV positive patients, alcoholics, those with history suggestive of malabsorption (irritable bowel disease, gastrectomy, ileal resection) and patients declining to participate were excluded from the study.

Data Collection
Information about demographic data, details of medical history, food habits, metformin dose and duration, other antidiabetic and concomitant medications, concomitant illnesses if any, investigations etc. was gathered from patients' medical records and also by enquiring with patients wherever required. This data was then entered into predesigned case record form.

Laboratory investigations
Fasting Sample was used for serum Vitamin B_{12} and HbA1c estimation. Vitamin B_{12} was measured by electrochemiluminescence immunoassay method (system: Cobas Elecsys 2010, Roche Diagnostics, Germany) while HbA1c was measured using high performance liquid chromatography (HPLC) method (System – HPLC, BIORAD, USA).

Data Analysis
Proportion of T2DM patients with normal levels of vitamin B_{12} and those with absolute and borderline deficiency of vitamin B_{12} was calculated. Absolute vitamin B_{12} deficiency was defined as ≤ 203 pg/ml, values of 203 -298 pg/ml were considered as borderline deficiency and values above >298 pg/ml as normal, based on cut-off values reported in previous studies.[6]

Statistical Analysis
For sample size calculation prevalence was considered to be 31%, error (d) and level of significance (α) were fixed at 10% and 5% respectively. This gave sample size of 82 patients.

Data analysis was performed with Statistical Package for Social Sciences (SPSS) version 22.0 U.S.A. The baseline characteristics and study variables are expressed as Mean ± S.D. or percentages. Association of vitamin B_{12} with various factors was studied by using chi square test for categorical variables and Mann-Whitney U test for continuous variables. Values of <0.05 were considered statistically significant.

Results

During study period, 220 T2DM patients were screened. The details of sample recruitment is shown in Figure 1.Demographic and baseline data (Table 1) showed that mean age of study population was 60.23 ± 9.20 years. The mean daily dose of metformin was 984.7 ± 185.7 mg. Majority of these (87.8%) patients received 1000 mg metformin daily. About 41.46 % (34/82) patients received combination therapy with metformin and glimepiride. Multivitamin supplements were used by 37.8% (31/82) patients. Vitamin B_{12} levels were normal in 54 patients (65.9%). Borderline deficiency was seen in 22 (26.82%) patients while absolute deficiency was seen in six (7.31%) patients. Combined borderline and absolute deficiency (≤298 pg/ml) was seen in 28 (34.1%) patients (Table 2). As shown in Table 3 and 4, age, gender, duration of diabetes, duration of metformin therapy, total daily dose of metformin, HbA1c value, use of sulfonylurea and use of H_2 antihistaminics was not significantly associated with vitamin B_{12} deficiency. Use of multivitamin led to significant decrease in frequency of vitamin B_{12} deficiency (16.1% vs. 45.1%, p=0.007).

Discussion

Metformin therapy is an integral part of pharmacological management of T2DM. Association of metformin treatment with reduction in serum vitamin B_{12} levels is now well established. Given the high prevalence of biochemical and metabolic deficiency of serum vitamin B_{12} in general Indian population [14] and paucity of studies in diabetic patients, estimating frequency of vitamin

B_{12} deficiency and finding variables associated with it in metformin treated T2DM patients in our setting was deemed essential. About 7.31% patients in the present study showed absolute vitamin B_{12} deficiency while combined absolute and borderline vitamin B_{12} deficiency was observed in 34.1% of patients. A cross-sectional study by Singh et al., [15] in Indian patients found definite vitamin B_{12} deficiency in 7.14% and possible vitamin B_{12} in 21.4% which supports our findings. Our results are more or less similar to several previous studies.[16-18] The prevalence of vitamin B_{12} deficiency varies widely in literature and thus true prevalence of vitamin B_{12} deficiency is difficult to estimate. This wide variation is mainly attributable to different cut-off values used to define vitamin B_{12} deficiency, different laboratory methods used to estimate serum vitamin B_{12} values, different inclusion and exclusion criteria, variation in mean total daily dose of metformin and total duration of metformin treatment.

The clinical significance of vitamin B_{12} deficiency in metformin treated diabetic patients is debatable. But several researchers have linked metformin induced vitamin B_{12} deficiency with serious clinical manifestations such as worsening of peripheral neuropathy, depression and cognitive impairment.[9-11] Estimation of serum vitamin B_{12} levels is most commonly used method for diagnosis of vitamin B_{12} levels because of relatively low cost. But measurement of serum vitamin B_{12} may not reliably detect deficiency. Hence measurement of serum homocysteine and/or methylmalonic acid has been recommended to confirm deficiency in asymptomatic high risk patients with low normal levels of vitamin B_{12}.[19]

The mechanisms underlying metformin induced vitamin B_{12} deficiency are not exactly known. Changes in small intestinal motility, bacterial overgrowth, consumption of vitamin B_{12} by bacteria, altered intrinsic factor,[20]and inhibition of calcium dependent ileal membrane absorption of vitaminB_{12}[21] have been suggested. An experimental study by Greibe et al., suggest metformin decreases circulating vitamin B_{12} by

increasing liver accumulation rather than causing malabsorption.[22] Oral administration of high dose vitamin B_{12} (1 - 2 mg daily) is as effective as intramuscular administration in correcting the deficiency, regardless of etiology.[19]

The present study also tried to assess various factors associated with vitamin B_{12} deficiency. We have evaluated association of age, gender, duration of T2DM, duration of metformin therapy, total daily dose of metformin, HbA1c level, use of H_2 antihistaminics, use of sulfonylurea and use of multivitamin. We found no significant association between vitamin B_{12} deficiency and age, gender, duration of T2DM, duration of metformin therapy, total daily dose of metformin, HbA1c level, use of H_2 antihistaminics or use of sulfonylurea. But patients taking multivitamin showed significant reduction in combined absolute plus borderline deficiency (16.1% vs. 45.1%, p=0.007). A study by Damio et al., found no association between vitamin B_{12} deficiency and age, gender, T2DM duration or metformin dose but use of H_2 antihistaminics and metformin duration $\geq$ 10 years were associated with biochemical vitamin B_{12} deficiency.[18]

A study by Aroda et al., did not find association between age, gender or prescription acid suppression use, the only significant predictor of vitamin B_{12} deficiency in this study was total metformin years of exposure.[23] Ko et al., found higher dose and long duration of metformin use to be significantly associated with vitamin B_{12} deficiency but age, gender, HbA1clevel, use of H_2 antagonist or sulfonylureas was not associated with vitaminB_{12} deficiency.[24]

Singh et al., report significant negative correlation between cumulative metformin dose and duration of metformin treatment.[15]A study by Nervo et al., showed no association of vitamin B_{12} levels with gender, HbA1c or dose of metformin but vitamin B_{12} levels were negatively associated with duration of metformin use and age and positively associated with daily intake of vitamin B_{12}.[17]A study by Pflipsen et al., showed no association of vitamin B_{12} deficiency with current or history of metformin use, H_2 antihistaminics use, age etc. In this study

multivitamin use on daily basis lowered prevalence ofvitamin B_{12} deficiency significantly.[16]

Overall our findings with regard to non-significant association between vitamin B_{12} deficiency and age, gender, duration of T2DM, HbA1c level, use of H_2 antihistaminics, use of sulfonylurea are supported by previous studies.

One of the important finding of the present study was protective effect of multivitamin against metformin induced vitamin B_{12} deficiency. The multivitamin preparation in our setting contained 15 micrograms of vitamin B_{12}. Similar protective effect of multivitamin or vitamin B_{12} supplements is reported by several previous studies.[6,16,17,25]

Several researchers have reported dose and duration of metformin therapy as an important predictor of vitamin B_{12} deficiency.[7,9,15,18,22,23,24,26] But present study could not find significant association between total daily metformin dose or duration of metformin therapy with vitamin B_{12}deficiency. The reason for this could be the mean daily dose of metformin in our study population was only 984.7mg/day and majority (87.8%) patient received 1000 mg/day which is far lower than maximum recommended dose of 2550 mg.[2] A systematic review by Liu et al., based on six randomized controlled trials found metformin dose >2000 mg was significantly associated with metformin induced reduction in serum vitamin B_{12} levels.[7] Likewise study by Akinlade et al., also showed metformin dose of >1000 mg to be significantly associated with vitamin B_{12} levels.[27]

There are some limitations to our study. First, we did not measure homocysteine or methylmalonic acid to confirm metabolic deficiency of vitamin B_{12} as these laboratory investigations are not routinely performed in our setting. Second, being cross-sectional study, we could only describe association between various factors and vitamin B_{12} deficiency but cannot prove causality. Third, this study involved small number of patients from a single tertiary care government hospital catering mainly to patients from low socioeconomic status and where oral hypoglycaemic treatment was confined to use of metformin and glimepiride. Therefore results of

this study may not be applicable to patients receiving treatment with large number of recently introduced drugs or different therapeutic regimens and belonging to higher socioeconomic strata.

Conclusions

In conclusion, absolute vitamin B_{12} deficiency was found in 7.31% and combined absolute plus borderline deficiency was found 34.1% of T2DM patients receiving mean metformin dose of 984.7 mg/day for more than a year. Use of multivitamin significantly reduced likelihood of vitamin B_{12} deficiency thus supporting its use in high risk patients receiving metformin. Further large scale longitudinal studies incorporating metabolic markers of vitamin B_{12} deficiency would be needed to clarify the role of early detection of metformin induced vitamin B_{12} deficiency and its impact on clinical manifestations.

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