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Association of Vitamin B_{12} with glycemic control in patients with Type 2 diabetes mellitus treated with metformin

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Abstract

Type 2 Diabetes Mellitus is one of the most common lifestyle metabolic disorders with its global prevalence increasing rapidly due to population aging, urbanization and associated lifestyle changes. Through many years, it has been predicted that long term consumption of metformin can cause B_{12} deficiency in patients of type 2 Diabetes Mellitus. This study aims to evaluate B12 levels in type 2 DM patients treated with metformin and to assess the correlation of Vitamin B12 with glycemic control, dose and duration of metformin consumed. The current study included total 90 subjects, which were divided into 3 different groups; Group 1 compromised of 30 healthy individuals, Group 2 included 30 T2DM patients with good glycemic control and Group 3 included 30 T2DM patients with poor glycemic control. Blood samples were collected from the three groups and FPG and PPG were estimated by fully automated auto analyser. HbA1c level was estimated by HPLC and vitamin B_{12} level was measured by *Chemiluminescence immunoassay.* Mean levels of FPG, PPG, HbA1c levels were significantly higher in group 3 compared to group 2($p < 0.0001$) and group 1($p < 0.0001$). We found negative correlation of B12 levels with HbA1c, dosage and duration of metformin consumed in group 2 and 3. Our study suggests that glycemic control, metformin dose, and duration of treatment all have a significant impact on the probability of developing metformin-associated vitamin B12 deficiency in T2DM patients

Keywords: Metformin, Vitamin B_{12}, Type 2 Diabetes Mellitus, HbA1c, Fasting Plasma Glucose, Postprandial Plasma Glucose

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1. Introduction

Type 2 Diabetes Mellitus is rapidly emerging as a global epidemic with Asia being on the topmost in the list, with China and India being the two topmost in the rank. The global Diabetes prevalence estimated in 2019 is estimated to be 463 million people, which is expected to rise to 578 million by 2030 which, according to the estimates, will further increase to 700 million by 2045 [1]. The three major defects towards which any cause associated to type 2 Diabetes Mellitus leads to, are, impaired insulin sensitivity (insulin resistance), diminished production of insulin secretion, and upregulated production of hepatic glucose production, all of these finally result in the characteristic feature of this disease, the hyperglycemic state [2]. Vitamin B12 (Cobalamin), a water-soluble vitamin plays a crucial role in pathways like haematopoiesis, neuro-cognitive functions and also in vascular system [3]. It's involved in methylation of homocysteine to produce methionine which is converted into its active form called S-adenyl Methionine (SAM) that acts as methyl donor in various other metabolic pathways [4]. Its deficiency causes accumulation of methylmalonic acid (MMA) which results in defective fatty acid synthesis of neuronal membrane [5]. Metformin hydrochloride, a biguanide, is the most commonly oral anti-hyperglycemic agent in most countries and it considered the foundational therapy for type 2 Diabetes Mellitus, due to its efficient glucose lowering capacity, economic, overall good drug safety profile (specifically zero risk of hypoglycaemia) [6]. Most guideline committees suggest metformin as the initial therapy to achieve glycemic targets [7] Metformin favours B12 deficiency through a not so fully established mechanism [8]. However, there are many proposed mechanisms for metformin induced B_{12} deficiency [9]. The majority of studies evaluated metformin-induced B12 deficiency in diabetic patients; relatively few studies have looked at the status of B12 deficiency in DM patients with good and poor glycemic control who are taking the metformin. Therefore, the present study is planned to analyse and compare B_{12} levels in T2DM patients with good Glycemic Control, poor glycemic control

and healthy individuals. We have also taken into consideration their dosage and duration of metformin consumption respectively.

2. Materials and Methods

2.1. Ethical Approval

The Institutional Ethics Committee of the MGM Medical College, Kamothe, Navi Mumbai has approved human participant enrolment and blood sample collection. [N-EC/2021/SC/02/14].

2.2. Study design and population

This prospective study was conducted through department of biochemistry and department of general medicine. A total of 90 subjects within the age group of 35 – 70 years were enrolled within three different groups. Group 1 compromised of 30 healthy individuals with normal glycemic control (HbA1c within the normal range i.e., 4-6%). Group 2 included 30 T2DM patients with good glycemic control (HbA1c levels with 6 – 7 %) and Group 3 included 30 T2DM patients with poor glycemic control (HbA1c levels more than 7%). The Institutional Ethical Clearance was obtained for the present study. Diagnosed Type -2 Diabetes Mellitus patients (as per WHO criteria) were enrolled in the study from Medicine department and apparently healthy individuals were enrolled from general population. Patients suffering from chronic disorders like Tuberculosis, HIV and liver Cirrhosis and on B12 supplementation were excluded from the study.

2.3. Biochemical Analysis

Blood samples were collected from three groups. Fasting Plasma glucose (FPG) and Post prandial plasma glucose (PPS) were estimated using hexokinase method. HbA1c levels were estimated using HPLC method. Serum Vitamin B_{12} levels were estimated Chemiluminescent immunoassay on ADVIA Centaur XPT Auto analyser.

2.4. Statistical Analysis

Data was analysed by SPSS version 23 and reported as mean ±SD. Comparison between two groups were assessed by student t –test. Correlation between two parameters was calculated by Pearson Correlation and Spearman's rho correlation.

3. Results and discussion

The comparison of biochemical parameters between group 1(healthy controls), group 2 (Subjects with good glycemic control) and group 3 (Subjects with poor glycemic control) is shown in Table 1. From the observations found from the study, the FPG, PPG and HbA1c levels were significantly higher in group 3 than in group 2 ($p < 0.0001$) and also the group 1(control group) ($p < 0.0001$). The FPG, PPG and HbA1c levels of group 2 were significantly higher than group 1 ($p < 0.0001$). This shows that there was a significant difference in the plasma glucose concentrations and has defined boundaries with respect to the monitoring of the disease between three groups. The mean concentrations of Vitamin B12 was significantly decreased in group 3 as compared to group 2 ($p < 0.001$) and Group 1(control group) ($p < 0.001$). We did not find significant difference between group 2 and 1 (($p > 0.05$). We found negative correlation between HbA1c levels and B12 levels in group 2 ($r = -0.228$, $p > 0.05$) & group 3 ($r = -0.38$, $p < 0.05$) respectively. M. Infante, M. Leoni, M. Caprio, A. Fabbri [9] had shown the similar results and has also mentioned that metformin therapy ≥5.0 years and age ≥ 65 years have more susceptibility towards development of B12 deficiency. C. Shivaprasad, K. Gautham, B. Ramdas, K.S. Gopaldatta, K. Nishchitha. [10] shows vitamin B12 levels were 200 pg/ml and between 200-300 pg/ml in 24.5% and 34.5% metformin users of T2DM patients respectively, which was higher than the non – metformin users T2DM patients (17.3% and 22.6%, respectively) ($p < 0.001$) . Further authors quantify metformin usage, in terms of "metformin usage index " (MUI) which was defined as the product of the daily metformin dose (mg) and its duration (years) divided by 1000. There was a significant correlation between MUI value of >5 and high risk of B12 deficiency. The highest risk was found in patients with MUI value >15, followed by MUI > 10. With a MUI value of less than 5, T2D patients were found to have the lowest risk. We noted negative correlation between dosage of metformin consumption and B12 levels in group 2 ($r = -0.272$, $p > 0.05$) & group 3 ($r = -0.533$, $p < 0.05$) respectively , this was supported by M.M. Hashem, A. Esmael, A.K. Nassar, M. El-Sherif. [11], M. Niafar, F. Hai, J. Porhomayon, N.D. Nader [12], M. Infante, M. Leoni, M. Caprio, A. Fabbri [9]. M.M. Hashem, A. Esmael, A.K. Nassar, M. El-Sherif. [11] reported similar negative correlation between higher doses of metformin and the cobalamin levels ($r = -0.52$, $p < 0.05$). This study also suggested that patients on metformin therapy are at a higher risk of developing diabetic peripheral neuropathy. M. Niafar, F. Hai, J. Porhomayon, N.D. Nader [12] had done a meta-analysis and reported that high dosage of metformin has significant impact on B_{12} levels and M. Infante, M. Leoni, M. Caprio, A. Fabbri [9] has observed that dosage of at least 6 months with a metformin dose of ≥ 1500 mg/ day has been observed to deplete B_{12} levels. Acid-suppressing medications like proton pump inhibitors for a long-term basis increases the risk of B_{12} deficiency. This implies that continuous high dosage of metformin causes clinical deficiency of B_{12} levels. We noted negative correlation between the duration of metformin consumption and the B12 levels in group 2 ($r = -0.14$, $p > 0.05$) & group 3 ($r = -0.42$, $p < 0.05$) respectively. N. Raizada, V.P. Jyotsna, V. Sreenivas, N. Tandon. [13] reported that when the duration of diabetes was taken into account, the usage of metformin was linked to significantly decreased serum Vitamin B12 levels, however higher serum Vitamin B12 levels were linked to longer diabetes duration; in this regard authors have reported that active efforts had been taken to exclude patients, who had been given Vitamin B12-containing supplements for any indication, but these preparations are available over the counter, and they cannot be sure that patients had never taken these medications earlier. D. Martin, J. Thaker, M. Shreve, L. Lamerato, K. Budzynska. [14] suggested that B12 screening needs to be done with patients on metformin therapy for more than 5 years, even if they are asymptomatic especially the elderly population since there B12 stores also get depleted [15].

Table 1. Comparisons of FPG, PPG, HbA1c and Vitamin B_{12} levels in Group 1, Group 2 and Group 3

Parameter	Group 1 (Control) (Mean ± SD)	Group 2 (T2DM with Good Glycemic Control) (Mean ± SD)	Group 3 (T2DM with Poor glycemic control) (Mean ± SD)
FPG (mg/dl)	85.89 ± 8.46	122.7 ± 19.80*	245.11 ± 63.64*, @
PPG (mg/dl)	98.56 ± 17.72	149.85 ± 30.05*	292.32 ± 74.44*, @
HbA1c (%)	5.2 ± 0.38	6.47 ± 0.29*	11.51 ± 2.26*, @
Vitamin B_{12} (pg/ml)	285.64 ± 118.95	278.90 ± 108.92#	147.58 ±52.81*, @

Group 1(control group) vs group II: $p < 0.0001$ * highly significant, $p > 0.05$ # Non significant
Group 1(control group) vs group III: $p < 0.0001$ * highly significant, $p > 0.05$ # Non significant,
Group II vs group III: $p < 0.0001$ @ highly significant, $p > 0.05$ # Non significant

Table 2. Correlation between HbA1c levels, dosage and duration of metformin consumption with serum vitamin B_{12} levels of patients enrolled in group 2 & group 3

S.No	Parameters	Group 2 r value	Group 3 r value
1.	Vitamin B12 vs HbA1c	-0.228#	-0.38*
2.	Vitamin B_{12} vs Dosage of metformin consumed per day (in mg)	-0.272#	- 0.533*
3.	Vitamin B_{12} vs Duration of metformin consumed (in years)	-0.14#	- 0.42*

$p < 0.05$ * significant, $p > 0.05$ # Non significant

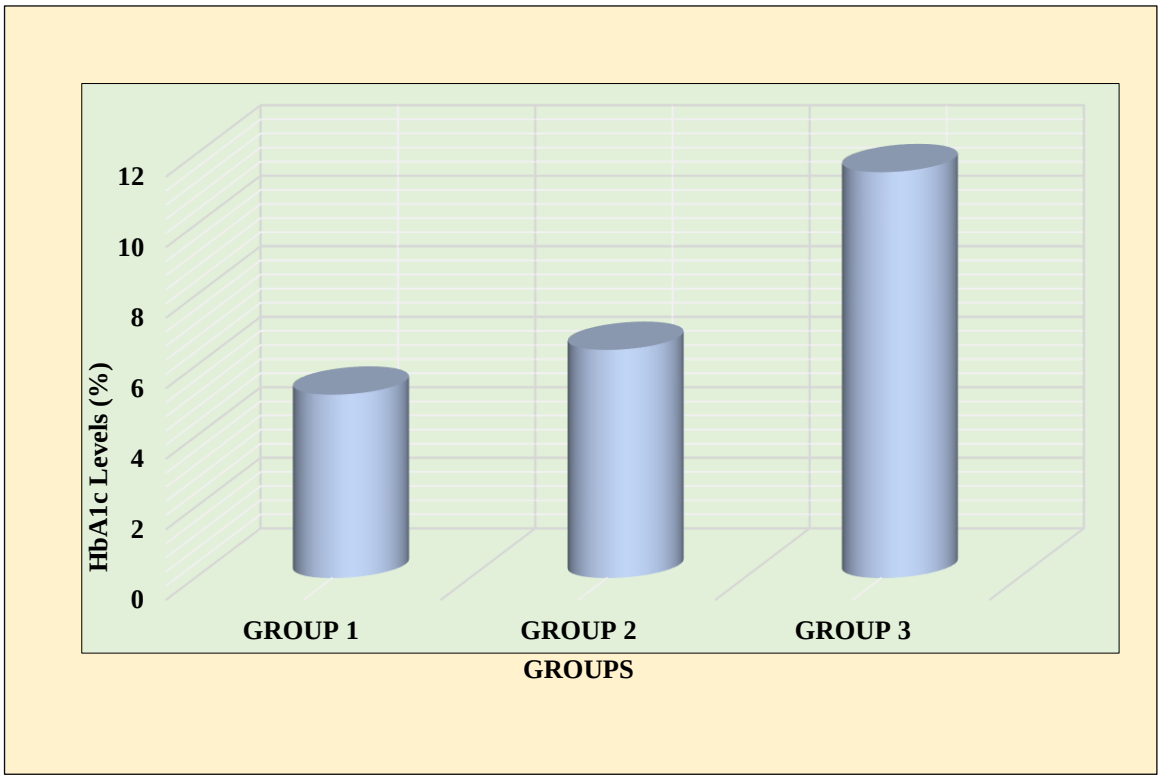

Figure 1. Shows comparative HbA1c levels amongst the groups

Group 1 (Control); Group 2(T2DM with Good Glycemic Control); Group 3 (T2DM with Poor glycemic control)

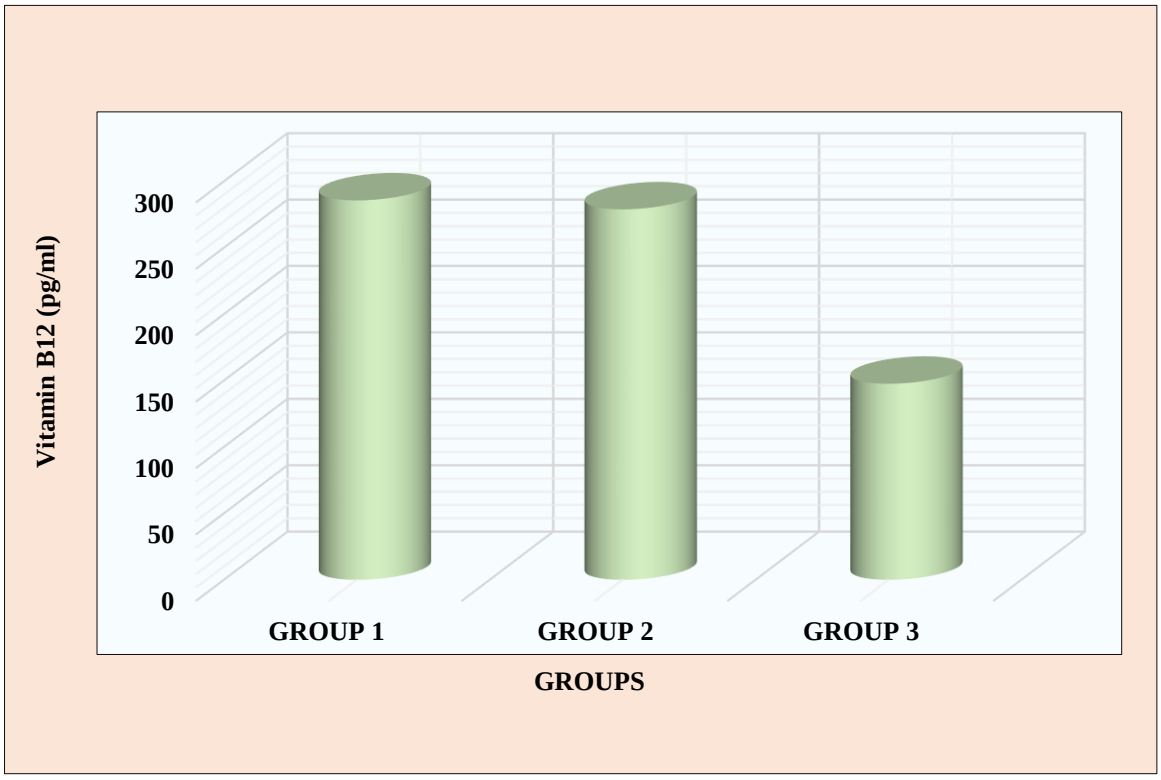

Figure 2. Shows comparative vitamin B_{12} levels amongst the groups

Group 1 (Control); Group 2(T2DM with Good Glycemic Control); Group 3 (T2DM with Poor glycemic control)

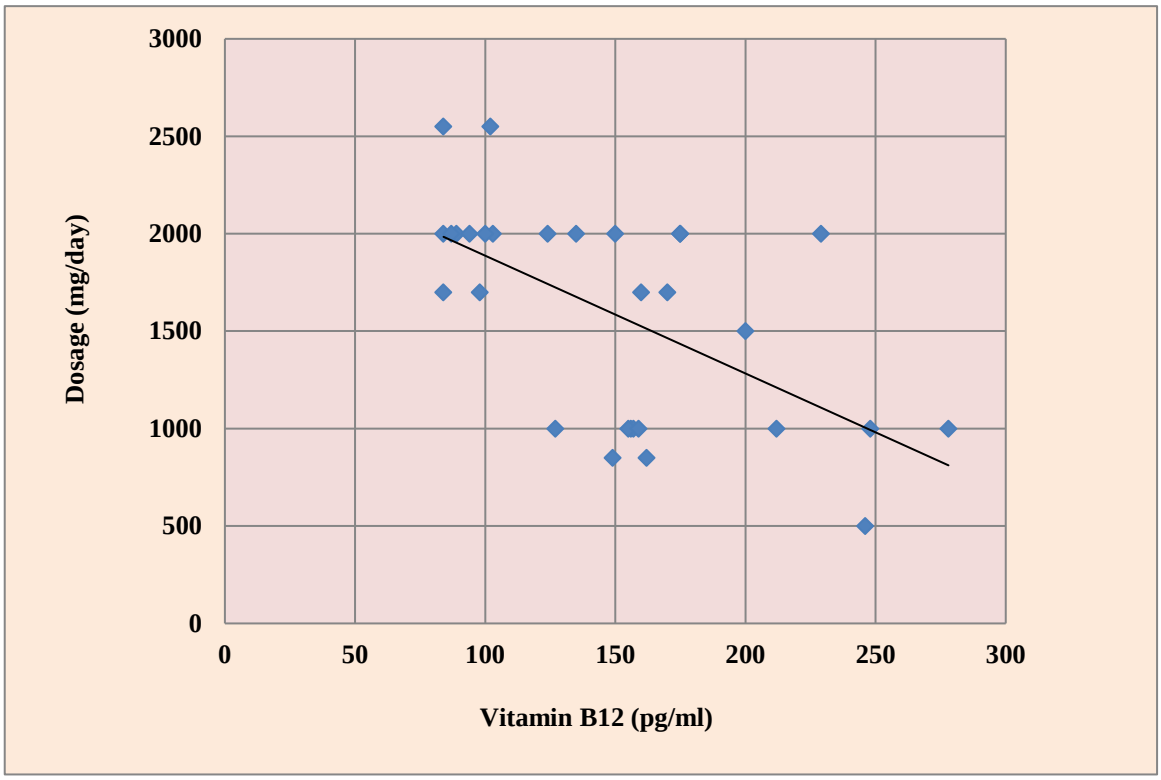

Figure 3. Correlation of dosage of metformin consumed per day with serum Vitamin B_{12} levels in group 3 (in pg/ml) ($r=0.533$, $p < 0.05$)

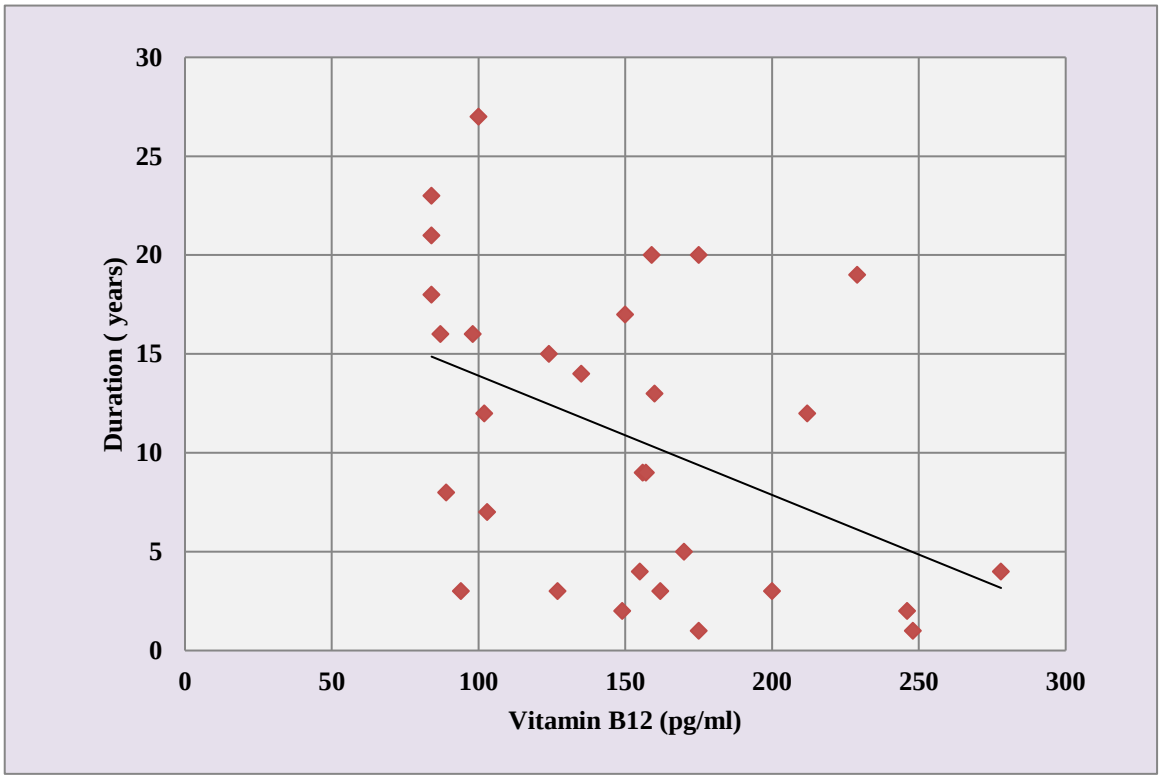

Figure 4. Correlation of Duration of metformin consumed (in years) with serum Vitamin B_{12} levels (in pg/ml) ($r=-0.42$, $p < 0.05$)

There are few proposed mechanisms for development of metformin induced B_{12} deficiency includes Hinderance in calcium-dependent binding of the IF- vitamin B_{12} complex to the cubilin receptor, Changes in the motility of small intestine which results in bacterial overgrowth which is supposed to inhibit IF-B_{12} complex absorption, Impaired enterohepatic circulation of B_{12} because of bile acid metabolism and reabsorption dysfunction, Over accumulation of B_{12} in hepatic tissue causing altered tissue distribution of B_{12}, Decreased secretion of IF from parietal cells may be due to underlying pathology [9]. The severity of this deficiency increases with increase in duration of metformin consumption and also with the dosage of metformin consumed. The high B12 deficiency seen in our study is not surprising, considering the B_{12} deficiency even in the healthy population of India, estimated to be around 33% to 67% [16] [17], major credit for this prevalence goes to the predominantly found vegetarian diet in India. [16] This should be taken into consideration while serving patients who needs regular monitoring for their diabetic condition. Since, metformin can cause B_{12} deficiency, which in long term, not good for neuro-cognitive functioning and might create hinderance in many of the metabolic pathways.

We found significant negative correlation of B12 levels with HbA1c, metformin duration and metformin dosage in T2DM with Poor glycemic control, however relatively weak non-significant correlation observed in T2DM with good glycemic control) (Table 2). In our study, T2DM patients with poor glycemic control were consuming comparatively high dosage of metformin per day from a long duration than T2DM patients with good glycemic control (Group2). This indicates that glycemic control, duration and dosage of metformin consumed have an impact on B12 levels. Second, we found no significant difference in Vitamin B12 levels in T2DM patients with good glycemic control compared to the control group. This could be due to tight glycemic control and these patients taking metformin for a shorter period of time and receiving a lower dose of metformin.

4. Conclusions

In our study we found a negative correlation of B12 levels with HbA1c levels, dosage of metformin consumed per day and duration of metformin consumed (in years) in T2DM patients with good and poor glycemic control. Thus our study suggests that glycemic control, metformin dose, and duration of metformin treatment, all have a significant impact on the probability of developing metformin-associated vitamin B12 deficiency. Therefore, Physicians should recommend that patients with T2DM especially older age group patients, who have poor glycemic control and are taking metformin need to have their vitamin B12 levels tested on a frequent basis to prevent further B12 deficiency induced complications.

Recommendations

The study included subjects from the local population which might not be the case for any other set of population. Also, the subjects who were in the group 1(control group) showed B_{12} levels towards the lower side of normal range, leading to the conclusion that the population irrespective of the type 2 Diabetes Mellitus can develop B12 deficiency, which will be supported, when their diet and habits are also taken into consideration. Therefore, further research can be extended by assessing B_{12} levels along with holo-TCII levels (holo-transcobalamin -II), MMA (methyl malonyl acid) and Hcy (Homocysteine) to provide better impact and to address the limitations.

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Conflict of interest

Authors have declared no conflict of interests.

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