International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Estimation of Vitamin B12 and Vitamin D Levels in Patients of Type-2 Diabetes Mellitus on Metformin Therapy

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Abstract: ***Introduction: Metformin is one of the first drug of choice in type-2 diabetes patients. However, long term use of metformin can lead to deficiency of vitamin B12 levels due to malabsorption. In addition, type-2 diabetes can be influenced in many ways by vitamin D deficiency. Here, we sought to estimate serum vitamin B12 and vitamin D levels in type-2 diabetes patients who were on metformin therapy. Materials and Methods: This was a case control study. A total of 60 patients were included for the study. 30 patients were on Metformin (CASES) and the other 30 were on other anti-diabetic drugs (CONTROLS). Serum vitamin B12 and vitamin D levels were measured by radioimmunoassay method. Results: This study clearly showed decreased vitamin B12 levels associated with metformin therapy. However, vitamin D deficiency was found among all the cases irrespective of metformin therapy. Conclusion: Type 2 diabetics on long term metformin therapy can develop vitamin B12 deficiency. Low levels of vitamin D was found in all type 2 diabetics, and was not specific for the type of anti-diabetic agent used. This could be probably due to lower levels of vitamin D in Indians and diabetics at large. Hence vitamin B12 supplementation is of benefit in type 2 diabetics on metformin, and vitamin D supplementation is needed in all Indian diabetics.***

Keywords: Type-2 diabetes mellitus, metformin, vitamin B12, vitamin D, deficiency

1. Introduction

Type-2 Diabetes Mellitus (T2DM) affects 8.3% of the global population and 9.09% of Indian population [1]. It adversely affects cardiovascular, renal and neurological systems of each person significantly increasing the morbidity and mortality [2].

Treatment of T2DM includes lifestyle changes, medications and insulin. Metformin, a drug from the class Biguanides, remains the first choice of treatment according to American Diabetes Association (ADA). In addition, it is one of the few anti-hyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality, which is a major cause of death in patients with type 2 diabetes [3].

Metformin acts through decreased glucose output , increased insulin mediated glucose uptake in the peripheral tissues and increases intestinal glucose utilisation. The most common side effect of Metformin includes gastrointestinal distress, soft stools and diarrhoea [4]. These gastrointestinal side effects lead to malabsorption of vitamin B12 in a dose and time dependent manner [5], [6]. However, the current and more likely explanation for metformin-induced vitamin B12 malabsorption and deficiency is that metformin has an effect on calcium-dependent membrane action in the terminal ileum [7]. On an average, 10% to 30% of the patients show malabsorptive deficiency of vitamin B12 [8,9] and this risk increases with age [10,11]

Recent studies suggest that there is an association between decreased vitamin D, calcium status and risk of T2DM. There is also an association between vitamin D deficiency and impaired glucose mediated insulin release.[12,13]. Vitamin D is produced endogenously when UV-rays initiate synthesis in the skin and it is also absorbed in the intestine from various natural and fortified food sources [14]. If gastrointestinal symptoms from Metformin use can lead to malabsorption of vitamin B12, it is probable that vitamin-D could also be malabsorbed.

This study focuses on estimation of vitamin B12 and D levels in patients with T_2DM on Metformin therapy.

2. Materials and Methods

Source of Data

This was a case control study. A total of 60 patients were included for the study. 30 patients were on Metformin (CASES) and the other 30 were on other anti-diabetic drugs (Sufonylureas, Acarbose and Pioglitazone) (CONTROLS) with age and sex matched, fulfilling the inclusion and exclusion criteria. The study was carried out in patients who visited OPD and got admitted in JSS Hospital from October, 2012 to October, 2014.

Inclusion Criteria:

AGE > 30 years

Type-2 DM patients who were on metformin therapy for more than 6 months.

Exclusion criteria.

1) AGE < 30 years
2) Type-1 DM patients
3) Patients who are on vitamin D supplementation
4) Patients who are on vitamin B12 supplementation
5) Patients who are on steroid therapy, oral contraceptive pills and diuretics
6) Pregnancy
7) Critically ill patients
8) Patients with tuberculosis
9) Patients with renal impairment

Method of collection of data

A detailed proforma was filled up for each patient, which included age, sex, IP and OP number, relevant present, past, personal history and clinical examination was done.

Anthropometric measurements like height and weight were measured and BMI was calculated for each patient in the study group. Venous plasma glucose was measured both fasting and prandial (120 min after a 75 g glucose load). HbA1C was measured by high performance liquid chromatography method.

Measurement of serum 25-(OH) vitamin D and serum vitamin B12

Serum vitamin B12 concentration was measured in samples stored at 20°C collected from both cases and controls. Serum vitamin B12 was measured by radioimmunoassay method.

Serum 25-hydroxyvitamin D concentration was measured in samples stored at 20°C collected from both cases and controls. Serum 25-(OH) vitamin D was measured by radioimmunoassay method.

Statistical methods applied

The results were analysed by appropriate statistical methods. All the statistical methods were carried out through the SPSS for windows (version 16.0).A p value <0.05 was considered as significant.

3. Results

Table 1: Age distribution among cases and controls
(*number in bracket indicates %)

Age Groups (years)	*Cases*	*Controls*	*Total*
<65	20 (33.3)	20 (33.3)	40 (66.6)
≥65	10 (16.6)	10 (16.6)	20 (33.3)
TOTAL	30	30	60

In this study, the study group constituted cases between the ages 32-90 years. Majority of the cases and controls were less than 65 years of age which constituted to 66.6% of the total group.

Table 2: Sex wise distribution of cases and controls

SEX	*Group*		*Total*
	Cases	*Controls*	
MALE	16	16	32 (53.3%)
FEMALE	14	14	28 (46.7%)
TOTAL	30	30	60 (100.0%)

In this study, 53.3% were males and 46.7% were females in both the groups.

Table 3: Mean age among cases and controls

	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
Age	Case	30	57.8000	16.15742	2.94993
	Control	30	58.7667	10.47389	1.91226

In this study, the mean age of the cases was 57.8±16.15 (SD) years and of the controls was 58.7±10.47(SD) years.

Table 4: BMI among cases and controls

BMI	**Cases**	**Control**	**Total**
<25	15 (50%)	12 (40%)	27 (45%)
>25	15 (50%)	18 (60%)	33 (55%)
Total	30	30	60 (100%)

In this study, 50% of the cases were with BMI>25 and 50% were with BMI<25, whereas in controls, 60% of them had BMI>25 and 40% had BMI <25%.

Table 5: FBS among cases and controls

	Group	N	Mean	Std. Deviation	Std. Error Mean
FBS	Case	30	120.6333	34.62358	6.32137
	Control	30	149.9000	42.47786	7.75536

In this study, mean FBS among the cases was 120.63±34.62(SD) mg/dl whereas that of controls was 149.9±42.47(SD)mg/dl.

Table 6: PPBS among cases and controls

	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
PPBS	Case	30	184.2000	56.09192	10.24094
	Control	30	215.3333	67.35564	12.29740

In this study, the mean PPBS among the cases was 184.2±56.09(SD) mg/dl,whereas among the controls was 215.33±67.35 (SD)mg/dl.

Table 7: HbA_1C among cases and controls

	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
HbA_1C	Case	30	6.7633	1.30503	.23826
	Control	30	7.1517	1.32811	.24248

In this study, the mean HbA1C among the cases was 6.76±1.3 (SD)%, whereas among the controls was 7.15±1.32 (SD) %.

Table 8: Serum creatinine among cases and controls

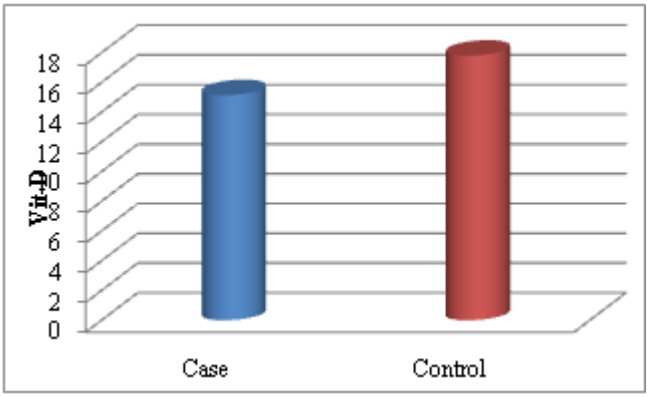
	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
S. Creat	Case	30	.8923	.14915	.02723
	Control	30	1.0300	.19853	.03625

In this study, the mean S. Creatinine among the cases was 0.89±0.14 (SD) mg/dl, whereas among the controls was 1.03±0.19 (SD) mg/dl

Table-9: Vitamin D levels among cases and controls

	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
Vit-D	Case	30	15.1497	4.73958	.86533
	Control	30	17.8077	7.05819	1.28864

p-value 0.092



Graph 1: Vitamin D levels among cases and controls

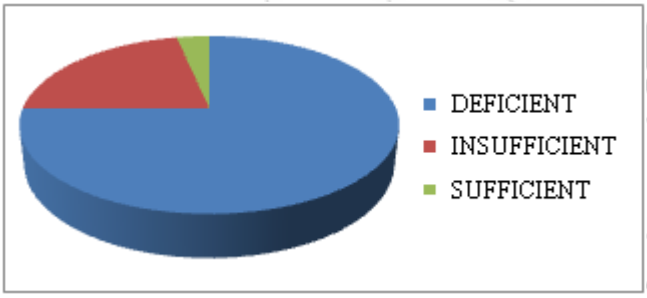
In this study, the mean vitamin D level among the cases was 15.14±4.73 (SD) ng/ml, whereas among the controls was 17.8±7.05 (SD) ng/ml.

Table 10: Classification of cases and controls in terms of vitamin D levels

Vitamin D (ng/ml)	Cases	Controls	Total
<20 (deficient)	25 (41.66)	20 (33.33)	45 (75)
20-29.9 (Insufficient)	5 (8.33)	8 (13.3)	13 (21.66)
>30 (Sufficient)	0 (0)	2 (3.33)	2 (3.33)
Total	30	30	60

***number in bracket indicates %**

In this study, among the cases 83.32% were vitamin D deficient, 16.66% were insufficient and 0% were sufficient. Among the controls, 66.66% were deficient, 26.6% were insufficient and 6.66% were sufficient. Also, as a whole, 75% were vitamin D deficient, 21.66% were insufficient and 3.33% were sufficient.

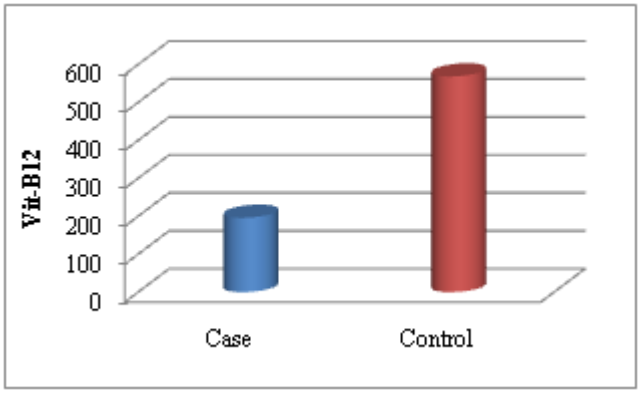


Graph 2: Vitamin D levels (Study population)

Table 11: Vitamin B12 levels among cases and controls

	Group	*N*	*Mean*	*Std. Deviation*	*Std. Error Mean*
Vit-B12	Case	30	195.9333	182.93016	33.39833
	Control	30	568.5000	459.33623	83.86294

p-value 0.00



Graph 3: Vitamin B12 levels among cases and controls

In this study, the mean vitamin B12 level among the cases was 195.93±182.9 (SD) pg/ml whereas among the controls was 568.5±459.33 (SD) pg/ml.

Table 12: Classification of cases and controls in terms of vitamin B12 levels

Vitamin B12(pg/ml)	Cases	Controls	Total
<210	21 (35)	7 (11.6)	28 (46.67)
≥210	9 (15)	23 (38.33)	32 (53.12)
Total	30	30	60

***number in bracket indicates %**

In this study, 70% of the cases were vitamin B12 deficient and 30% were sufficient whereas , among the controls, 23.2% were vitamin B12 deficient and 76.8% were sufficient.

4. Discussion

In regard to vitamin B12 levels, this study clearly shows a decrease associated with Metformin therapy. This result was expected because it is well documented in the literature that Metformin does cause a decrease in vitamin B12 levels in a time- and dose-dependent manner. The current and more likely explanation for metformin-induced vitamin B12 malabsorption and deficiency is that metformin has an effect on calcium-dependent membrane action in the terminal ileum [7].

Vitamin B12 malabsorption is poorly recognized and not screened for or treated prophylactically by the majority of physicians who prescribe metformin. In this study, the mean vitamin-B12 levels among the cases were 195.93±182.9 (SD) pg/ml whereas among the controls was 568.5±459.33 (SD) pg/ml with 'p' value 0.00 showing major significance. Metformin induced vitamin B 12 deficiency can present as peripheral neuropathy in diabetic patients which can be mistaken for diabetic peripheral neuropathy. It is recommended that serum vitamin B12 level should be checked every year in those patients who are on metformin therapy. Perhaps a more practical and cost-effective approach would be to give every patient on metformin an annual 1000 microgram injection of vitamin B12 which is sufficient to cover vitamin B12 needs for at least a year.

In our study, among the cases 83.32% were vitamin D deficient, 16.66% were insufficient and 0% were sufficient. Among the controls, 66.66% were deficient, 26.6% were

insufficient and 6.66% were sufficient. Also, as a whole, 75% were vitamin D deficient, 21.66% were insufficient and 3.33% were sufficient

In this study, the mean vitamin D levels among the cases was 15.14±4.73 (SD) ng/ml, whereas among the controls was 17.8±7.05 (SD) ng/ml.p value 0.092. According to a south Indian study, vitamin D deficiency was present in 83% of type 2 diabetes individuals and 82% of normal individuals. So both south Indian type2 and non type 2 diabetes individuals are equally deficient in vitamin D [15]. Hence, the effect of metformin therapy on vitamin D levels cannot be commented.

Our study had few limitations most notably; the sample size was small and may not truly reflect the whole group. Long standing diabetics are known to have gastro-intestinal disturbances related to autonomic neuropathy which can cause vitamin B12 deficiency which could not be ruled out. We, also could not rule out few other causes that can hamper its absorption.

5. Conclusion

Type 2 diabetics on long term metformin therapy were found to have significantly low levels of vitamin B12 when compared to type 2 diabetics on other anti-diabetic drugs. On the contrary, lower levels of vitamin D were found in all type 2 diabetics, and were not specific for the type of anti-diabetic agent used. This could be probably due to lower levels of vitamin D in Indians and diabetics at large. Hence vitamin B12 supplementation is of benefit in type 2 diabetics on metformin and vitamin D supplementation is needed in all Indian diabetics.

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