Original Research Article

Effect of metformin on serum level of vitamin B$_{12}$ and folate in patients of type-2 diabetes mellitus

Rajesh Meena*, Ramesh Kumar, Atul Meena, Om Prakash Meena, Lokesh Meena, Arun Meena

Department of Medicine, RNT Medical College, Udaipur, Rajasthan, India

*Correspondence:
Dr. Rajesh Meena,
E-mail: meenarajesh614@gmail.com

ABSTRACT

Background: Several types of DM are caused by a complex interaction of genetic and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycaemia include- reduced insulin secretion, decreased glucose utilization, and increased glucose production. Objective of the current study was to study the effect of metformin on the level of vitamin B$_{12}$ and folate in patients of type 2 DM.

Methods: This is hospital based study before and after metformin therapy randomized controlled trial was conducted in medicine ward of M. B. hospital, Udaipur. Baseline serum vitamin B$_{12}$ and folate level of all patients were measured and treatment with metformin 500 mg twice a day was given for 6 months. After 6 months serum vitamin B$_{12}$ and folate level of all patients were re-evaluated.

Results: There was a significant positive correlation (r=0.824, p<0.001) between decrease in vitamin B$_{12}$ and decrease in folate level after metformin treatment. When analysis for change in vitamin B$_{12}$ is compared with change in MCV values after 6 months, negative correlation (r=-0.08, p>0.05) was obtained. A non significant correlation (r=-0.08, p>0.05) with change in level of serum folate and change in MCV values or haemoglobin level was obtained.

Conclusions: Low serum vitamin B$_{12}$ level is associated with longer duration and higher dose of metformin use. Routine determination of vitamin B$_{12}$ level in patients with type 2 DM on high dose of metformin and those with prolonged use of metformin might help in identifying patients that would benefit from vitamin B$_{12}$ supplements.

Keywords: Type 2 diabetes mellitus, Metformin, Vitamin B$_{12}$, MCV, Serum folate

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders that share the phenotype of hyperglycaemia. Several types of DM are caused by a complex interaction of genetic and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycaemia include- reduced insulin secretion, decreased glucose utilization, and increased glucose production.[1-3] The metabolic dysregulation associated with DM causes secondary changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system.

The two broad categories of DM are- type 1 and type 2. Type 1 DM is the result of complete or near-total insulin deficiency. Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production.[1] Random is defined as without regard to time since the last meal. Fasting is defined as no caloric intake for at least 8 hours.

Treatment

The goals of therapy for type 1 or type 2 DM are to; eliminate symptoms related to hyperglycaemia, reduce or

eliminate the long-term microvascular and macrovascular complications of DM and allow the patient to achieve as normal a lifestyle as possible. We mainly focus our attention to treatment of type 2 DM.[1]

Treatment of type 2 diabetes mellitus

Advances in the therapy of type 2 DM have generated oral glucose-lowering agents that target different pathophysiologic processes in type 2 DM. Based on their mechanisms of action, glucose-lowering agents are subdivided into agents that increase insulin secretion, reduce glucose production, increase insulin sensitivity, and enhance GLP-1 action. Examples are- biguanides, α-glucosidase inhibitors, dipeptidyl peptidase iv inhibitors, insulin secretagogues: sulfonylureas, insulin secretagogues: non-sulfonylureas, thiazolidinediones, bile acid sequestrants, insulin, GLP-1 receptor agonists, amylin agonists etc.[1,4-7] Metformin, which reduces hepatic glucose production and improve peripheral glucose utilization slightly. Metformin activates AMP-dependent protein kinase and enters cells through organic cation transporters. Metformin reduces fasting plasma glucose and insulin levels, improves the lipid profile, and promotes modest weight loss.[1,7-9] The initial starting dose of 500 mg once or twice a day can be increased to 1000 mg bid. An extended-release form is available and may have fewer gastrointestinal side effects (diarrhoea, anorexia, nausea, metallic taste). Because of its relatively slow onset of action and gastrointestinal symptoms with higher doses, the dose should be escalated every 2-3 weeks based on SMBG measurements. Metformin is effective as monotherapy and can be used in combination with other oral agents or with insulin. The major toxicity of metformin, lactic acidosis is very rare and can be prevented by careful patient selection. Metformin should not be used in patients with renal insufficiency [GFR <60 ml/min], any form of acidosis, CHF, liver disease, or severe hypoxemia. Metformin should be discontinued in patients who are seriously ill, in patients who can take nothing orally, and in those receiving radiographic contrast materials. Another study "increased intake of calcium reverses vitamin B_{12} malabsorption induced by metformin" has been done in which a comparative study design was employed using 2 groups (metformin and control). A total of 21 patients with type 2 diabetes received sulfonylurea therapy; 14 of these 21 patients were switched to metformin. Monthly serum total vitamin B_{12} measurements and holotranscobalamin (B_{12}-TCII) were performed. After 3 months of metformin therapy, oral calcium supplementation was administered.[10]

Aims and objectives

Primary objective of current study is to assess the effect of metformin on the level of vitamin B_{12} and folate in patients of type-2 DM in the population of Southern Rajasthan. Other objectives of the current study were; to assess and compare the serum level of vitamin B_{12} before and after metformin therapy, to find out the correlation of serum level of vitamin B_{12} with haemoglobin and MCV values, to assess and compare the serum levels of folate before and after metformin therapy and to find out the correlation of serum level of folate with haemoglobin level and MCV values.

METHODS

This is a hospital based randomized cross-sectional observational study. Each and every eligible case of type 2 diabetes attending medicine OPD, RNT medical college hospital, Udaipur from 1 November 2018 to 31 October 2019 and were admitted to wards of M. B. Government hospital and thereafter came for regular follow-up. The study was conducted after taking due approval from the institutional ethical committee.

Study population

Patients of type 2 diabetes, who are not taking metformin, not having deficiency of vitamin B_{12} and/or folate and not taking any medication, which can affect the level of vitamin B_{12} and/or folate and who are not on any diet, which significantly affect the level of vitamin B_{12} and/or folate. Total 146 patients of type 2 diabetes those were not on metformin therapy and those were not taking vitamin B_{12} and/or folate and those were not taking any medication, which affect the level of vitamin B_{12} and/or folate and those were not taking any diet which significantly affect the level of vitamin B_{12} and/or folate.

Inclusion criteria

Inclusion criteria for current study were; patient should be a known case of type 2 diabetes and not on metformin treatment. Patient does not have deficiency of vitamin B_{12} and/or folate. They are not taking any medication which affects the level of vitamin B_{12} and/or folate and they are not taking any diet, which significantly affects vitamin B_{12} and/or folate level.

Exclusion criteria

Exclusion criteria for current study were; presence of any hepatic, renal or renovascular dysfunction/ failure in subject. Patient is already deficient in vitamin B_{12} and/or folate or is taking vitamin B_{12} and/or folate supplements or if taking any medication which affects the level of vitamin B_{12} and/or folate. Patient is taking such diet, which significantly affects vitamin B_{12} and/or folate level and unwillingness to participate in study.

Diet and medication affecting vitamin B_{12} and/or folate level

Diet affecting vitamin B_{12} level like fish, shellfish, meat, poultry egg, fortified breakfast cereals, fortified soy products, fortified nutritional yeasts etc. Medications affecting vitamin B_{12} level; alcohol, aminosalicylic acid, chloramphenicol, colchicines, cholestyramine,

cholestipol, Neomycin, nitrous oxide, zidovudine, phenytoin, phenobarbitone, primidone, H2 receptor antagonists, proton pump inhibitors etc. Diet affecting serum folate level like leafy vegetables, legumes, egg yolks, baker's yeast, some breakfast cereals, sunflower seeds, liver and liver products etc. Medications affecting serum folate level like trimethoprim, pyrimethamine, methotrexate, sulphonamide, valproic acid etc.

Statistical analysis

Descriptive analysis, Chi square, Pearson's Correlation Coefficient were applied as per requirement and p value was calculated with help of SPSS ver 20.

Sample size

Sample size was calculated using formula give below:

$$n = (Z\_\alpha^{\wedge}(2) \; P(1-P)) / E^{\wedge}2$$

Where; E=margin of error was taken to be 10%. P=prevalence of type 2 diabetes mellitus was 18% and at 95% confidence level thus $Z\_\alpha = 2.96$. Thus the calculated sample size was 146 patients.

RESULTS

At the onset of the study 146 patients were enrolled. After 6 months on follow up 8 patients did not turn up and hence their data were not available. Rest of the 138 patients were evaluated and analyzed below: The study population is divided into three age groups with mean age 44 years. We had 11 male patients (7.9%) & 13 female (9.4%) in 36-40 years age group; 42 male patients (30.4%) & 35 female (25.3%) in 41-45 years age group; 23 male patients (16.6%) & 14 female (10.1%) in 46-50 years age group. In total 76 (55.1%) patients were male and 62 (44.9%) were female in study population (Table 1). Mean and standard deviation of various parameters of both the visits of patients i.e. 0 months and then at 6 months after taking metformin are shown in (Table 2).

Table 1: Distribution according to age and sex.

Age groups (years)	Males N (%)	Females N (%)	Total
36-40	11 (7.9)	13 (9.4)	24 (17.4)
41-45	42 (30.4)	35 (25.3)	77 (55.8)
46-50	23 (16.6)	14 (10.1)	37 (26.8)
Total	76 (55.1)	62 (44.9)	138 (100)

Peripheral blood film which was done at the start of the study was found normocytic for all of the patients. After 6 months of metformin therapy 6 patients showed Macrocytic changes in peripheral blood film (Chi value = 4.25) (Table 3). Correlation between change in serum vitamin B_{12} level and change in Hemoglobin concentration in our study. We found significant

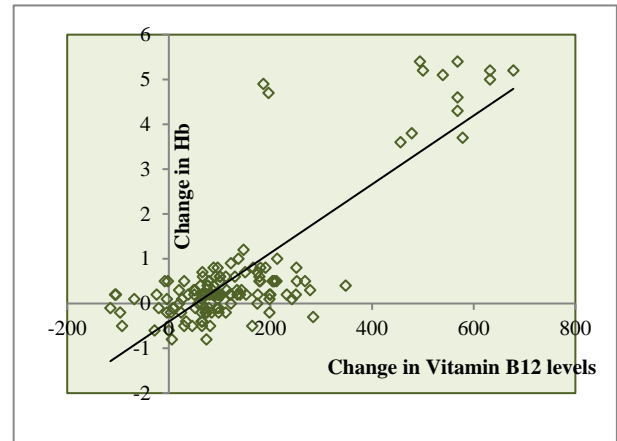correlation ($r=0.824$, $p<0.001$) between these two parameters (Figure 1).

Figure 1: Correlation between change in serum vitamin B_{12} and change in hemoglobin level after taking metformin.
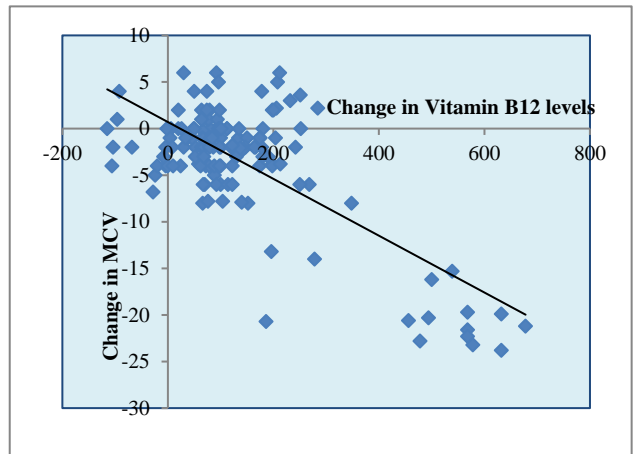

Figure 2: Correlation between change in serum vitamin B_{12} and change in MCV after taking metformin.
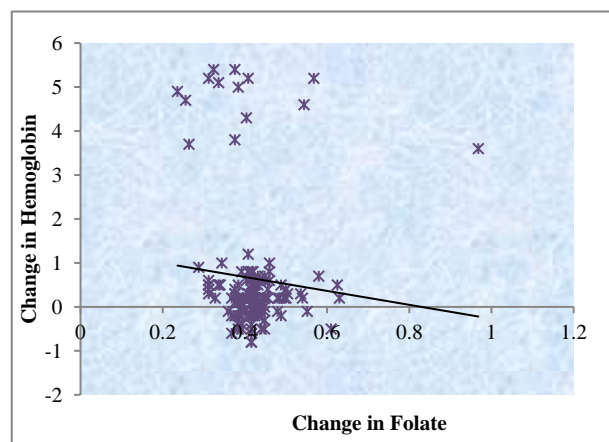

Figure 3: Correlation between change in serum folate and change in hemoglobin concentration after taking metformin.

Correlation between change in serum vitamin B_{12} level and change in MCV in our study on follow up. We found a significant negative correlation (r=-0.727, p<0.001) between these two parameters (Figure 2). Correlation between change in serum folate level and change in hemoglobin concentration after 6 months of metformin. We found a non-significant correlation between these two parameters (r=-0.08, p>0.05) (Figure 3). Correlation between change in level of serum folate and change in MCV after 6 months of metformin. We found a non-significant correlation between these two parameters (r=0.08, p>0.05) (Figure 4).

DISCUSSION

During 6 months of follow up 8 out of 146 patients did not turn up so their data were not available and thus lost in follow up. Therefore effective study populations were 138 patients.

Age

In current study 138 patients were classified in three different age groups like 36-40 years (17.4% or N=24), 41-45 years (55.8% or N=77), 46-50 years (26.8% or N=37). Out of 138 patients 76 (55.1%) were found to be males and 62 (44.9%) were females.

Vitamin B_{12} and hemoglobin

In current study we found a significantly positive (r=0.824, p<0.001) correlation between change in vitamin B_{12} and change in hemoglobin concentration before and after metformin treatment. It shows that change in level of vitamin B_{12} positively correlates with change in concentration on hemoglobin.

Vitamin B_{12} and MCV

In the study we found significant but negative correlation (r=-0.727, p<0.001) between these two parameters. It states that when there is a change in level of vitamin B_{12}, it inversely affects the level of MCV significantly. In one of the previous study done by Wulffele MG et al 850 mg thrice a day of metformin treatment was administered to patients for 16 weeks and this was found to be associated with a decrease in serum vitamin B_{12} level by 14% (p<0.001).[11]

In another long term study done by Jolien de Jager et al treatment was done with metformin 850 mg thrice a day for 52 months was given.[12] This study added that the negative effect of metformin treatment on concentration of vitamin B_{12} increases over time and was associated with a 19% decrease in its concentration at the end of 52 months.

Table 2: Mean±SD of various parameters before and after metformin group patients.

Parameters	Before 6 months of metformin	After 6 months of metformin	P value	Significance
Vitamin B_{12} (pg/ml)	868.1±84.9	730.74±191.6	<0.001	Highly significant
Folate (ng/ml)	10.4±1.85	10.02±1.78	>0.05	Non significant
MCV (fl)	82.15±2.34	85.63±5.86	<0.001	Highly significant
Hb (g/dl)	14.91±0.6	14.26±5.86	<0.001	Highly significant

Table 3: Peripheral blood film changes observed during study period.

Peripheral blood film	Before	After
Normocytic	138	132
Macrocytic	00	6
Microcytic	00	00

Chi value=4.25; p=0.039

Folate and hemoglobin

In our study we found a non significant correlation (r=-0.08, p>0.05) between change in serum folate level and change in hemoglobin concentration. It was found that change in level in folate does not significantly affect the concentration of hemoglobin.

Wulffele MG et al administered 16 weeks treatment with metformin 850 mg thrice a day and found that the treatment was associated with a decrease in folate

concentration of 7% (p=0.024) which was non significant.[11]

Folate and MCV

We found a non significant correlation between change in serum folate level and change in MCV (r=-0.08, p>0.05). It states that change in serum folate doesn't significantly affect the level of MCV. Sahin et al reported that after treatment, metformin use was associated with an increase in levels of Hcy by 2.36 micromol/l and decreases in folate and vitamin B12 concentrations by -1.04 ng/ml and -20.17 pg/ml.[12] Metformin also significantly decreased body weight. In controls, there was no change in Hcy, folic acid, vitamin B12, TG, LDL, total-C, HbA1c, insulin, or HOMA levels. Homocysteine change did not correlate with insulin, folate, or vitamin B12 changes in the metformin and rosiglitazone groups.[13-16] Pongchaidecha et al reported that the plasma Hcy levels showed no significant difference (p=0.544) among patients in the metformin group compared with those in

the non-metformin group (10.6$\pm$5.8 mol/l vs. 10.4$\pm$4.0 mol/l). There was a significant difference (p=0.011) in the levels of serum vitamin B12 among patients in the metformin group and among those in the non-metformin group (318.0$\pm$192.2 pg/ml vs. 434.3$\pm$300.7 pg/ml). However, there was no significant difference (p=0.090) in serum folic acid levels between patients in the metformin and those in the non-metformin group (8.8$\pm$5.1 ng/ml vs. 7.7$\pm$3.8 ng/ml). The plasma Hcy levels showed a significant correlation with the duration of metformin treatment (p=0.014) and the amount of metformin received (p=0.015) with the Spearman correlation coefficient of 0.260 and 0.258 respectively.[14-15]
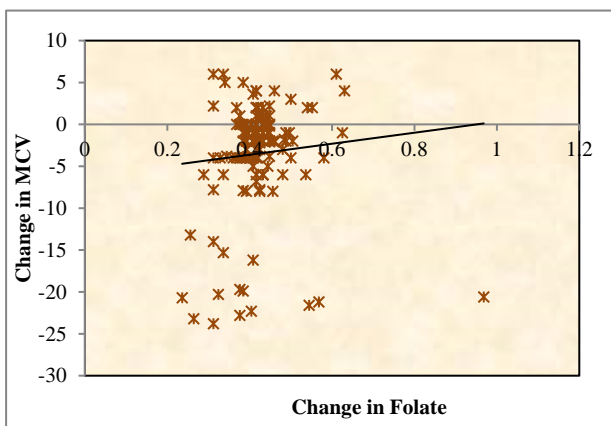

Figure 4: Correlation between change in serum folate and change in MCV after taking metformin.

CONCLUSION

It is concluded from this study that low serum vitamin B$_{12}$ level is associated with longer duration and higher dose of metformin use. Therefore, routine determination of vitamin B$_{12}$ level in patients with type 2 diabetes mellitus on high dose of metformin and those with prolonged use of metformin might help in identifying patients that would benefit from vitamin B$_{12}$ supplements.

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