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RESEARCH ARTICLE

Effect of Anti-Diabetic Drugs on Vitamin B12 and Mineral Levels and their Role in Inflammation in type II Diabetes Mellitus (T2DM)

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Abstract

Objectives: The purpose of the present study is to know the association between antidiabetic drugs and serum B12, and minerals like calcium, phosphorus, magnesium and zinc to understand their role in inflammation in type 2 diabetes mellitus. **Methods:** The sample size is 500 blood samples of both genders which was divided into 3 groups: A, B, and C. Group A on metformin of daily dosage 500mg consisted of 200 subjects, Group B on other anti-diabetic drugs of daily dosage 2 mg consisted of 200 subjects. Both groups A and B had no other complications from diabetes. Group C included controls, consisting of 100 healthy individuals with no history of diabetes. **Findings:** When compared between two drug(metformin and sulfonylureas) groups, the percentages of Serum B12, calcium, phosphorus, magnesium and zinc in both male and female groups were significantly decreased and consequently, inflammatory markers like leptin, IL-6, hsCRP, TNF-α were significantly increased in metformin group rather than who were on metformin and other associated drug group. **Novelty :** Decreased serum B12 levels in type 2 diabetes mellitus who are on metformin drug represents the potential trigger for inflammatory process which leads to micro and macrovascular complications when compared to sulfonylurea drug group.

Keywords: Diabetes mellitus; Cobalamine; Calciumdependent process; Antioxidants; Inflammatory process

1 Introduction

India and China are the pinnacle epicentres in Asia displaying essential rising Type 2 Diabetes Mellitus worldwide epidemics[1,2]. Diabetes is a multifactorial disease, and metformin is the primary line of drug suggested. Metformin is an extensively used drug that has many effects associated with glucose metabolism and diabetes-associated complications through enhancing peripheral insulin sensitivity[3,4] . Physiologically,

metformin reduces hepatic glucose production, but no longer all of its results may be defined through this mechanism, as there's growing proof of its crucial function withinside the gut. At the molecular level, the findings range depending at the dosage of metformin and the duration of treatment. Metformin has been proven to act through each AMP-activated protein kinase (AMPK)-structured and AMPK-independent mechanisms, through inhibition of mitochondrial respiration, however additionally possibly through inhibition of mitochondrial glycerophosphate dehydrogenase, and a mechanism related to the lysosome.

Vitamins and minerals exert important implications for the risk of diabetes mellitus, as well as its progression and complications. The best recommendation should be to consume adequate amounts of foods that contain vitamins and minerals in sufficient quantity to ensure an adequate nutritional status [(5)]. Vitamin B12 is one of the nine water-soluble vitamins that are important for the healthy functioning of the body. Vitamin B12 is an essential micronutrient needed for optimal hematopoietic, neurocognitive, and cardiovascular function. Because the effects are seen across a wide range of functions, symptoms of vitamin B12 deficiency can sometimes be very ambiguous. Intake of vitamin B12 begins with ingestion and then digesting it through saliva. Once in the intestine, the vitamin B12 bound to protein in the food is released by the acids present. B12 can bind to intrinsic factor. Once vitamin B12 is bound to Intrinsic factor, it is stable enough to enter the gut where it can be absorbed. Two very useful tests to differentiate between vitamin B12 deficiency and folate deficiency are methyl malonyl-CoA (MMA) and homocysteine (hcy). The increase in levels of methyl malonyl CoA and homocysteine is thought to be the root cause of any symptoms that accompany a Vitamin B12 deficiency.

Minerals play an important role in glucose homeostasis, so understanding the effects of vitamin and mineral deficiencies and the potential benefit of supplementation for the prevention and/or treatment of type 2 diabetes mellitus (DM) is relevant. Vitamins have antioxidant properties that protect them. B12 deficiency in diabetics has been linked to oxidative stress and resulting hyperhomocysteinemia. Calcium has also been studied to play an important role in insulin secretion and absorption of B12 in the ileum, a calcium-dependent process [(6)]. Patients receiving metformin have diminished B12 absorption and low serum total vitamin B12 and TCII-B12 levels because of a calcium-dependent ileal membrane antagonism, an effect reversed with supplemental calcium.

T2DM is considered an oxidative stress disease, the burden of which is amplified by micronutrient deficiencies. Many studies point to the role of micronutrients in delaying the harmful effects of diabetes. However, studies on the relationship between drugs used to treat T2DM and their effects on micronutrients are lacking. Metformin use over time has been linked to biochemical B12 deficiency. Routine testing of vitamin B12 levels in metformin-treated patients should be considered. At 5 years (4.3 vs 2.3%; P =.02), low B12 (203 pg/mL) occurred more frequently in the metformin group, but not at 13 years (7.4 vs 5.4%; P =.12).At 5 years (19.1 vs 9.5%; P.01) and 13 years (20.3 vs 15.6%; P =.02), the metformin group had a higher prevalence of combined low and borderline-low B12 (298 pg/mL).Metformin use was associated with an increased risk of B12 deficiency (odds ratio, B12 deficiency/year metformin use, 1.13; 95% confidence interval, 1.06–1.20).Anemia prevalence was higher in the metformin group, but did not differ by B12 status. Neuropathy prevalence was higher in the metformin group with low B12 levels.

2 Methodology

This study was conducted through collaboration among the physiology department of a teaching medical institute, the medicine department, and the Genetics Lab. Ethical approval for this study was received 007/02/2019-/IEC/SMHC-and the study was conducted from March 2019 to February 2021**.** Based on WHO and ADA guidelines for the screening of type 2 diabetes mellitus, the plasma glucose criteria, the Fasting Plasma Glucose (FPG) value or the 2-h Plasma Glucose (2-h PG) value during a 75-g Oral Glucose Tolerance Test (OGTT), or A1C criteria. The present study recruited 500 participants between 30 and 65 years of age. Patient pool who has been recently diagnosed as type 2 diabetics on metformin usage for more than 12 to 18 months. The study design was divided into 3 groups: A, B, and C. Group A consisted of 200 subjects with type 2 diabetes only on metformin with a daily dosage of 500 mg/day, and Group B consisted of 200 subjects with type 2 diabetes who were on both metformin with a daily dosage of 500 mg/day and other anti-diabetic drugs of around 2 mg/day. Both groups A and B had no other complications from diabetes. Group C included controls, consisting of 100 healthy individuals with no history of diabetes. The study included type 2 diabetics on metformin for more than a year as cases and age-matched non-diabetic healthy volunteers as controls. People who are on vitamin B12 supplements, calcium supplements, or proton pump inhibitors No complications from liver diseases, renal diseases, gastrointestinal disorders, thyroid diseases, or parathyroid disorders. Strict vegetarians, alcoholics, and smokers were excluded. A detailed clinical history, drug history, dosage of the drug, and B12 supplementation were documented in a structured proforma. The blood samples were collected from the subjects via vein puncture for fasting plasma glucose, glycated haemoglobin (HbA1c), and serum (creatinine, Zn, and magnesium (Mg)) were determined and quantified. Whole blood samples were collected into EDTA-coated vacutainers for quantification of glycated haemoglobin

(HbA1c), zinc, and magnesium in the blood. Fasting and postprandial plasma glucose were estimated using the glucose oxidase-peroxidase method. Serum magnesium was estimated on an automatic bioanalyzer (Beckman Coulter, Inc). 19,20 Serum zinc was quantified using an Abcam's Zinc Quantification Kit and absorbance was checked at 560nm. Serum calcium, phosphorus, and vitamin B12 were measured with the Chemiluminescent Microparticle Immuno Assay, which is a modified and advanced form of the Enzyme Linked Immuno Sorbent Assay (ELISA) technique. We ensured that the study complies with international ethical norms according to the Helsinki Declaration-Ethical Principles for Medical Research Involving Human Subjects (World Medical Association et al. 1964).

2.1 Estimation of inflammatory markers

2.1.1. Interleukin 6 (IL6)

IL6 levels were quantified in the serum of the subjects by the sandwich ELISA method. A highly specific IL-6 antibody (1:1000 dilution) was coated on the microtiter plate overnight at 40C. The IL-6 present in the sample (1:500 dilution) would bind to the specific antibody and subsequently bind to the biotinylated anti-IL-6 secondary antibody (1:8000 dilution), later incubated with Streptavidin-HRP (1:10000 dilution) solution for 60 minutes. The plate was developed by incubating with TMB Substrate solution for 15 minutes and stopped by adding 1.25mM H_2SO_4 and the OD was read at 540nm.

2.1.2. Leptin and highly sensitive C-reactive protein

Leptin and hs-CRP in the serum were measured using the standard kit (Cal Biotech) by adopting the sandwich ELISA principle.

2.1.3. Tumor necrosis factor α (TNF α)

TNF α was measured in subjects' serum using a commercial kit (Diaclone SAS), and the protocol was the same as described in the section (IL-6).

All data were statistically analysed and expressed as mean standard deviation. The mean was analysed by one way ANOVA (with a student T-test for comparison with controls). A Pearson correlation test was done to see the relationship between control subjects and diabetics.

3 Results and Discussion

3.1 Biochemical assays

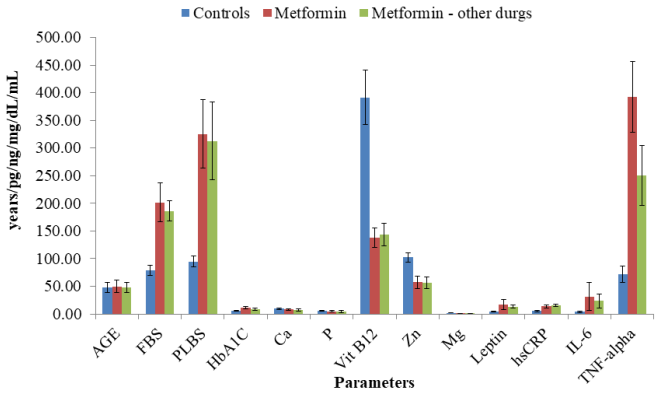

Fig 1. A comparison of FBS, PLBS, HbA1C, calcium, phosphorus, vitamin B12, inflammatory markers (leptin, CRP), cytokines (TNF, IL6), and micronutrient (zinc and magnesium) levels among Males in Metformin group, Other antidiabetic drug group and control group

Figure 1 represents the levels of different biomarkers in male subjects on metformin alone and with metformin and other antidiabetic drugs. There is a significant increase in blood glucose levels and inflammatory biomarkers (leptin, hsCRP, TNF- , and IL6), but there is also a loss of vitamin B12, zinc, and magnesium in both metformin alone and metformin combined with other diabetic drugs when compared to control subjects, with metformin alone showing the least aggravated condition of vitamin B12 loss.

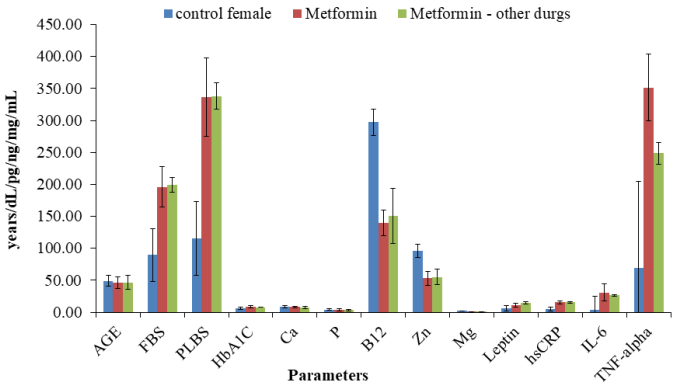

Fig 2. A comparison of FBS, PLBS, HbA1C, calcium, phosphorus, vitamin B12, inflammatory markers (leptin, CRP), cytokines (TNF, IL6), and micronutrient (zinc and magnesium) levels among Females in Metformin group, other antidiabetic drug group and control group.

Observations made from Figure 2clearly indicate the long-term side effects of metformin alone and with metformin-other antidiabetic drugs in female subjects. There is a significant rise in the blood glucose levels and inflammatory biomarkers (leptin, hsCRP, TNF-a and IL6), but there is also a loss of vitB12, zinc, and magnesium in both metformin alone and metformin with other diabetic drugs in comparison to the control subjects. Among them, subjects with metformin alone show a little aggravated condition of VitB12 loss, which would lead to various pathies in the future due to vitB12 deficiency.

Table 1. The standard reference range.

I	FBS	< 100 mg/dl
II	PPBS	< 180 mg/dl
III	HbA1c	< 5.7% normal
	Prediabetic	-5.7- 6.4%
	Diabetic	-6.5%
IV	Vitamin B12	- 200 - 900 pg/ml
V	Calcium	- 9.3 - 9.9 mg/dl
VI	Magnesium	- 1.7-2.2 mg/dl
VII	Zinc	- 75 - 125 mg/dl
VIII	Phosphorous	- 2.5 - 4.5 mg/dl
IX	TNF-alpha	- 177 pg/ml
X	hsCRP	- 0.2 to 10mg/ml
XI	IL-6	- 5.15 pg/ml
XII	Leptin	- 7.3 ± 3.7ng/ml

Figure 3 elucidates the comparative analysis of different biochemical parameters in male and female subjects who are under metformin and metformin-other diabetic drug treatments for T2DM. The inflammatory marker TNF- was significantly higher in male subjects on metformin alone compared to male subjects on metformin and other diabetic drugs, and similarly, the levels

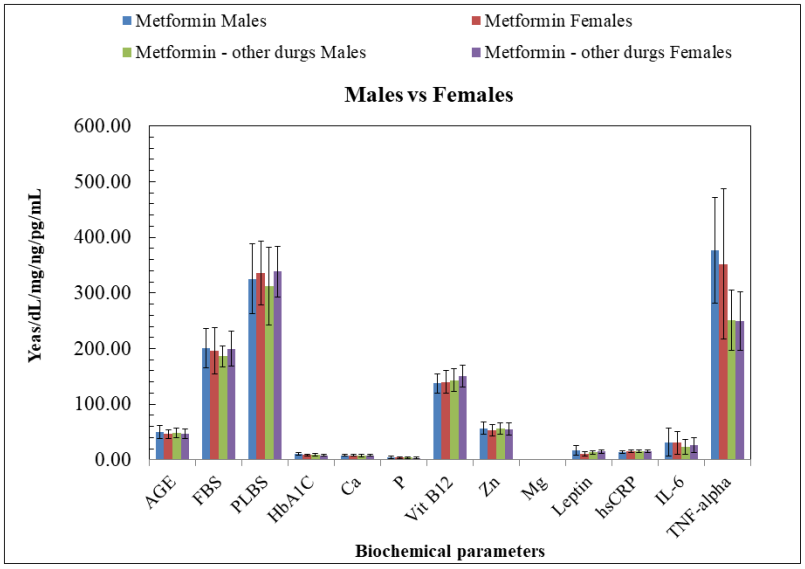

Fig 3. Elucidates the comparative analysis of different biochemical parameters and inflammatory markers in male and female subjects who are under metformin and metformin-other diabetic drug treatments for T2DM.

were significantly higher in female subjects on metformin alone and other diabetic drugs.

An escalating proportion of research on animals and clinical trials suggests that the key function of metformin is to decrease hepatic glucose production, mainly by suppressing gluconeogenesis [7,8]. The inhibitory effects on hepatic gluconeogenesis can be due to changes in enzyme activities or suppressed hepatic uptake of gluconeogenic substrates supported by research evidence. Cellular uptake of metformin is facilitated by the chief expression of OCT1 (organic cation transporter 1) in the hepatocytes

A rational accumulation of metformin in the liver could be higher compared to other tissues, leading to high micromolar concentrations in the per portal area· Research over a certain time period indicates metformin's action is targeted around the intestines, by reducing the net glucose uptake and enhancing anaerobic glycolysis in enterocytes, causing an increased release of lactic acid in the liver. The molecular level findings vary depending on the dosage of metformin and the duration of treatment, with some differences between acute and chronic administration. Metformin has been shown to act via both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms; by inhibition of mitochondrial respiration and also perhaps by inhibition of mitochondrial glycerol phosphate dehydrogenase.

Absorption of VitB12 is a multifactorial process and depends on factors like Haptocorrin (HC), also commonly known as the R-protein secreted by the salivary glands of the oral cavity, which binds to vitamin B_{12}, which is an intricate and necessary mechanism to protect against the acidic environment of the stomach. In this complex, Haptocorrin attaches to VitB12 to create a complex, and this Haptocorrin–B_{12} complex passes via the pylorus to the duodenum, where HC is cleaved from the complex in the presence of pancreatic proteases. Intrinsic factor (IF) is a glycoprotein secreted by parietal cells of the gastric mucosa and the IF binds to the free vitamin B_{12} to form the VitB_{12}–IF complex. Then, the vitamin B12 is transported to the ileum by the ileal receptor (i.e., Cubam) which comprises the Cubilin and Megalin complex, and takes them up into the circulation via endocytosis-mediated absorption. Hence, as ileal vitamin B12 absorption is a calcium-dependent process, in our study, patients with type 2 diabetes developed a marked reduction in serum vitB12 and Ca levels in patients treated with metformin alone for T2DM [9].

Singh et al. in their studies showed T2DM patients on metformin treatment had lower levels of B12. Similar findings were reported by Roy et al. that patients on metformin had a lower level of B12. According to the DPPOS study, an increased risk of B12 deficiencyis shown to have an association with metformin. A recent study from India has shown an association between metformin use and B12 levels. Similar studies by Den Elen et al on B12 deficiency in older adults with prolonged proton pump inhibitors and H2 blockers have also reported hypocobalinemi· A meta-analysis was done by Chapman et al. on the B12 lowering effect of metformin, which took approximately 6 weeks to 3 months after commencing metformin. All these studies were in correlation with our studies showing decreased B12 levels with oral anti-diabetic drug usage. We found B12

levels had significantly decreased by about 82% in the metformin group and 62% in the associated anti-diabetic drug group when compared with controls [10]. According to Reinstatler et al., metformin therapy is associated with a higher prevalence of biochemical B12 deficiency.

Bauman et al. reported that there is a 10–30% decline in VitB12 levels in people who are on metformin due to diminished ileal b12 absorption as it is a calcium-dependent process [11]. In our study, we included screening for calcium levels in all the groups as it has a major role in the signalling cascade where it is shown to have 38% decreased levels of calcium in the metformin group, 58% in the associated antidiabetic drug group, and it was normal in the control group. In our present study, we could find a widespread association between phosphorus levels and different groups. A study done by Ellen et al. suggested that there was no effect on phosphorus levels in the subjects who were on metformin. Anwar et al. subjects with T2DM showed higher levels of fasting blood sugars, postprandial blood sugars, and glycated haemoglobin levels, which are correlated with a reduction in serum levels of zinc and magnesium, showed a significant inverse correlation with glycaemic control when compared to controls [12,13]. In our study, we included screening for both serum zinc and magnesium levels in all the groups and we tried to associate the levels of zinc and magnesium in the metformin group, other antidiabetic drug groups, and control group. We have also witnessed that there is an association between pro inflammatory markers with the levels of FBS, HbA1C, and micronutrients in subjects with metformin alone and also with other diabetic drugs [14,15]. There is upregulation of pro inflammatory markers in the metformin group when compared with the control group. Further research is required to find the association between anti diabetic drugs and levels of pro inflammatory markers in T2DM [16] .

4 Conclusion

Finally, the study has led to an important proof against the antidiabetic drugs commonly advised to Type 2 Diabetes Mellitus patients for controlling their blood glucose levels, but instead they are facing the inevitable consequence of being VitB12 deficient and its associated manifestations. As diabetes itself is a chronic inflammatory disease and diabetics on metformin have decreased serum B12 as a major side effect. Which itself is a potent stimulator for inflammation which leads to microvascular and macrovascular complications like retinopathy, nephropathy, microvascular neuropathy, ischemic heart disease, peripheral vascular disease, and cerebrovascular disease due to decreased scavenging of reactive oxygen species, by increasing homocysteine induced oxidative stress when compared between both the drug groups, serum B12 has significantly decreased in metformin group than sulfonylurea group. Future large and well-designed studies on screening for vitamin B12 and minerals deficiency, and optimal supplementation dose among type 2 diabetic patients are warranted to help guide formulation of guidelines in diabetes clinical care.

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