STUDY TO EVALUATE RELATIONSHIP OF SERUM VITAMIN B12 WITH METFORMIN THERAPY IN DIABETIC PATIENTS OF JHARKHAND.

Diabetology

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ABSTRACT

Background: Vitamin B12 is a water soluble vitamin required for normal functioning of the brain and nervous system besides formation of blood. It has been reported that diabetic patients taking metformin has been said to be an important pharmacological cause of vitamin B12 deficiency. Hence the aim of study is to correlate serum B12 level with oral hypoglycemic agents(OHA) like metformin and sulfonylurea, and insulin therapy.

Material and methods: This is a cross-sectional study and was carried out in a group of 136 randomly selected diabetic patients in the Department of Medicine at Rajendra Institute of Medical Sciences, Ranchi between January 2016 to December 2017. Inclusion criteria were diabetic patients on metformin,sulfonyl urea and insulin or combination of any two. A control of 40 people from normal population was also taken. Vitamin B12 estimation was done by electrocheiluminescence immunoassay in all 136 patients.

RESULT: 84 (61.77%) of diabetic patients selected were on metformin treatment, 22(16.77%) of diabetic patients were on sulfonyurea and the rest 30(22.05%) of diabetic patients was on insulin. 16(11.76%) patients having vitamin B12 levels less than 180 pg/ml lie in the metformin group and 6(4.41%) patients in the probably low range (180-200 pg/ml) also lie in the metformin group. Significant difference in mean vitamin B12 levels in metformin group (412±193.16 pg/ml) and non-metformin group (549.27±80.40pg/ml), (p = 0.0261). Significant difference between mean value of vitamin B12 levels treated with metformin >12 months (305.87±151.94 pg/ml) and mean values of vitamin B12 treated with metformin < 12 months (553.50±147.14 pg/ml) (p < 0.0001).

CONCLUSION There was significant difference between the mean values of vitamin B12 levels in the metformin group and in the sulfonylurea and insulin groups (p value 0.0261 and 0.0751 respectively). There was no statistical significant difference between the mean values of vitamin B12 in the diabetic population and the control from normal population (p value=0.6398). We concluded that vitamin B12 levels was definitely lowered with long term use of metformin.

KEYWORDS

OHA (oral hypoglycemic agents)

INTRODUCTION: Vitamin B_{12}, also called cobalamine, is a water soluble vitamin with a key role in the normal functioning of the brain and nervous system besides formation of blood. It is involved in the metabolism of every cell of human body affecting DNA synthesis and regulation including fatty acid synthesis.

Intrinsic factor is crucial for the normal absorption of vitamin B_{12}. Intrinsic factor is produced in the gastric parietal cells of the fundus and body of the stomach. The IF-cobalamin complex enters the ileal cell, where intrinsic factor is destroyed and cobalamine transported to portal blood through transcobalamine II. Pernicious anemia is an autoimmune disease in which parietal cells of the stomach responsible for secreting intrinsic factor is destroyed.

Cobalamin vitamer includes: cyanocobalamine, hydroxycobalamin and cofactors forms like adenosylcobalamin, methylcobalamin.

The dietary reference intake for an adult ranges from 2 to 3 microgram per day. Body stores are of the order of 2-3mg, sufficient for 3-4 years if supplies are completely cut off. The normal range is>180pg/ml.

Two main cobalamine transport receptors exist in human plasma. TCI derived from neutrophils play a role in transport of cobalamine to the liver for excretion in bile. TCII is synthesized by liver transports cobalamine in blood.

Vitamin B_{12} is found in foods that come from animals, including fish, meat, poultry, eggs, dairy and dairy products.

Vitamin B_{12} is used to regenerate folate in the body. When folic acid is available, all known B_{12} related deficiency syndrome normalizes.

It has been reported that diabetic patients taking metformin has been said to be an important pharmacological cause of vitamin B_{12} deficiency. It is estimated that 10% to 30% of patients undergoing metformin therapy develops evidence of vitamin B_{12}deficiency (Victor A. Hoffbrand, 2012)[1].

The responsible mechanism for vitamin B_{12} deficiency in metformin users has been controversial; proposed contributors have included competitive inhibition or inactivation of Cb1 absorption, alterations in intrinsic factor levels, altered bacterial flora, gastrointestinal motility, and interaction with the cubulin endocytic receptor. Patients on metformin have low B_{12} levels because of a calcium dependent ileal membrane antagonism.

Low B_{12} levels due to prolonged metformin use can cause or exacerbate diabetic peripheral neuropathy.

Type2 DM more typically develops with increased age (Alwin C. Powers, 2012)[2].

Criteria for the diagnosis of diabetes mellitus (ADA criteria):
1. Symptoms of diabetes plus random blood glucose concentration ≥11.1mmol/l (200mg/dl)
2. Fasting plasma glucose ≥ 7.0 mmol/l (126mg/dl)
3. HbA1c >6.5%
4. Two hour plasma glucose>11.1 mmol/l (200mg/dl) during an oral glucose tolerance test.

(American Diabetes Association, 2011)[3]

Metformin representative of the biguanide class of agents, reduces hepatic glucose production and improves peripheral glucose utilization. Metformin reduces fasting plasma glucose and insulin levels, improves the lipid profile, and promote modest weight loss. Metformin should not be used in patients with renal insufficiency (GFR<60ml/min), any form of acidosis, CHF, liver disease or hypoxemia (Andrès E, Noel E, Goichot B, 2002)[4].

MATERIAL AND METHODS:

This is a cross-sectional study and was carried out in a group of 136 randomly selected diabetic patients in the Department of Medicine at Rajendra Institute of Medical Sciences, Ranchi between January 2016 to December 2017.

Inclusion criteria were diabetic patients on metformin, diabetic patients on metformin plus other OHA and insulin, diabetic patients on metformin with insulin, diabetic patients with features of neuropathy, myelopathy or dementia. Patients with diabetic nephropathy were excluded. A control of 40 people from normal population was also taken. After a detailed history, clinical examination and investigations, 136 patients of diabetes mellitus was subjected to vitamin B_{12}assay.

Vitamin B_{12} estimation was done by electrocheiluminescence immunoassay. Electroluminescence (ECL) is a process in which reaction of highly reactive molecules are generated from stable state electrochemically by an electron flow cell forming highly reacted species on a surface of a platinum electrode producing light (Kobrynski L et al, 1996)[5].

The most important one in measuring vitamin B_{12} concentration is the competitive principle. The competitive principle is applied to low molecular weight molecules. It uses antibodies (intrinsic factor) for vitamin B_{12} labeled with ruthenium complex.

1. Serum and plasma (heparinized) are the recommended samples.
2. Observe the following recommendations for handling, processing and storing blood samples:

The diagnosis of vitamin B_{12} deficient patients was based on mean corpuscular volume, hematocrit, the presence of megaloblastic cells in bone marrow aspirates, and by vitamin B_{12} RIA. A non-parametric estimate at the 95% confidence level yields the following ranges:

Units	Normal rage	Intermediate range	Deficient range
pg/mL	200-914	180-200	≤ 180
pmol/L	148-676	133-148	≤ 133

OBSERVATION:
Maximum numbers of patients belong to 51-60 years age group (55.88%). The mean age of the diabetic population was 57.05 years. The male to female ratio in this group was 5.8:1. There were 130(95.58%) of diabetic patients selected of type II diabetes and 6(4.41%) patients was of type I diabetes. In the metformin group (n=84), there were 13.23% patients, who were vegetarian and 86.76% patients were non-vegetarian.

When diet considered as an confounding factor to affect vitamin B12 levels, was statistically analysed in the metformin group, the p value came out to be 0.5645 which showed non significant affection of diet on vitamin B12 level. 84 (61.77%) of diabetic patients selected were on metformin treatment, 22(16.77%) of diabetic patients were on sulfonyurea and the rest 30(22.05%) of diabetic patients was on insulin. 124(91.17%) of diabetic patients on peripheral blood picture show normocytic normochromic pattern, 6(4.41%) shows microcytic microchromic pattern and the rest 6(4.41%) shows macrocytic macrochromic pattern.

TABLE – 1. PATIENTS IN THE NORMAL AND LESS THAN NORMAL CUT OFF VALUES OF VITAMIN B12 IN THE STUDY.

	Normal range (200-914 pg/ml)	Borderline (180-200 pg/ml)	Low (< 180 pg/ml)
Metformin group	62	6	16
Sulfonylurea group	22	0	0
Insulin group	30	0	0
Total	114 (83.82%)	6 (4.41%)	16 (11.76%)
Mean value	518.96 pg/ml	194.66 pg/ml	158.25 pg/ml

Table.1 shows that 16 patients having vitamin B12 levels less than 180 pg/ml lie in the metformin group & 6 patients in the probably low range (180-200 pg/ml) also lie in the metformin group.

TABLE – 2 : COMPARISON OF VITAMIN B12 IN THE METFORMIN AND SULFONYLUREA GROUP

Group	No. of cases	Mean vitamin B12 value (pg/ml) ± SD	'p' value
Metformin group	84	412±193.16	0.0261
Sulfonylurea group	22	549.27±80.40	

Table.2 shows significant difference in mean vitamin B12 levels in metformin group (412±193.16 pg/ml) and non-metformin group (549.27±80.40pg/ml), (p = 0.0261).

TABLE – 3: COMPARISON OF VITAMIN B12 IN THE METFORMIN AND INSULIN GROUP

Group	No. of cases	Mean vitamin B12 value (pg/ml) ± SD	'p' value
Metformin group	84	412±193.16	0.0175
Insulin group	30	539±86.70	

Table.3 shows significant difference in mean vitamin B12 levels in

metformin group (412±193.16 pg/ml) and in insulin group (539±86.70 pg/ml), (p = 0.0175).

TABLE – 4 :COMPARISON OF VITAMIN B12 LEVELS BETWEEN THE DIABETIC POPULATION AND NORMAL CONTROL

Group	No. of cases	Mean vitamin B12 value (pg/ml) ± SD	'p' value
Diabetic	136	462.22±171.79	0.6398
Control group	20	480.50±43.40	

Table.4 shows non-significant difference in mean vitamin B12 levels in diabetic population (462.22±171.79pg/ml) and in normal control (480.50±43.40pg/ml) (p = 0.6398). Significant difference between mean value of vitamin B12 levels treated with metformin >12 months (305.87±151.94 pg/ml) and mean values of vitamin B12 treated with metformin < 12 months (553.50±147.14 pg/ml) (p < 0.0001).

TABLE – 5 : CORRELATION OF MEAN VITAMIN B12 VALUES WITH DOSE OF METFORMIN

Dose of Metformin (gm)	No. of patients	Mean vitamin B12 levels with S.D.
0.5	26	490.84±26.89
1.0	32	506.81±171.82
1.5	8	333.75±173.27
2.0	14	166.00±19.61
2.5	4	158.50±6.36

Table.5 shows proves significant negative correlation (r = - 0.65, p value <0.01) between dose of metformin and serum vitamin B12 levels.

DISCUSSION:
Out of 136 diabetic patients, 84 patients were on metformin, 22 patients were on sulfonylurea and 30 patients were on insulin. The male to female ratio in the study is 5.8:1.In this study there was no difference in the vitamin B12 levels with reference to gender of the patient. The study by Koshy AS et al. (2012)[6] also did not show any significant difference in vitamin B12 levels between male and female. In this study, 84 patients were kept on metformin therapy, of which 16 patients (19.04%) had significantly low vitamin B12 levels (<180pg/ml) and 6 patients (7.14%) had low levels of vitamin B12 (180-200pg/ml).

There was significant difference (137.27pg/ml, 95% CI 17.04 to 257.50, p value= 0.0261) between the mean values of vitamin B12 levels in metformin group (412±193.16 pg/ml) and in the sulfonylurea group (549±80.40 pg/ml). There was similar difference (127 pg/ml, 95% CI 23.06 to 230.93, p value= 0.0175) between the mean values of vitamin B12 levels in metformin group (412±193.16 pg/ml) and in the insulin group (539±86.70).These results were very consistent with the previous studies of Shihong Chen et al (2012)[7] who in his observational found significant lowering of vitamin B12 levels with metformin.

Carpentier et al (1976)[8] noticed in their study the difference in mean values of vitamin B12, in patients who were taking metformin and in those not taking metformin.

A cross sectional study in Brazil by Monique Nervo et al (2010)[9] showed results of low vitamin B12 (< 125 pmol/L) in 10 patients and possibly low (125 to 250 pmol/L) in 53 patients taking metformin.

Leif Sparre Hermann et al (2004)[10] conducted a cross sectional study and observed that metformin treated patients had an deteriorated vitamin B12 status as compared to other treatments in type 2 diabetes .

Pflipson et al (2009)[11] concluded in his study the prevalence of metabolically confirmed vitamin B12 deficiency in type II Diabetic population who was on metformin therapy.

There was no significant statistical difference (-18.27 pg/ml, C.I = -95.64 to59.08, p value = 0.6398) between the mean values of vitamin B12 levels in the diabetic population (462.22±171.79 pg/ml) and in the mean values of control from normal population (480.50±43.40 pg/ml). This result is explained by only marginal to small lowering of serum vitamin B12 in metformin cases.

In this study, there was no significant difference in the mean values of the haematological parameters between the metformin and the non-metformin groups (p > 0.05). 91.17% of the patients peripheral picture shows normocytic normochromic pattern, 4.41% shows macrocytic macrochromic pattern and 4.41% shows microcytic microchromic pattern. Carpentier et al (1976)[26] in their study also did not find any difference in the values of red blood cell counts, corpuscular volumes or haemoglobin concentration. The lack of haematological alteration may be explained by the fact that the vitamin B12 deficiency was not present for a sufficient period of time.

In the present study, metformin treated patients were divided on the basis of duration of therapy and a clear association of decreased vitamin B12 levels was observed with increase in the duration of metformin therapy. There was significant difference in the mean values of vitamin B12 (305.87±151.94 pg/ml) who had taken metformin for more than one year and in the mean values of vitamin B12 (553.50±147.14 pg/ml) who had taken metformin for less than one year. p value was <0.0001 (significant). This observation is very well related to the study by Monique Nervo et al (2010)[9] who found low vitamin B12 levels in 6.9% patients (out of 144 total diabetic patients) and probably low values in 36.8% patients (out of 144 total diabetic patients) who have been taking metformin for more than one year.

The association of duration of metformin use with serum vitamin B12 levels was, (r = -0.128, p = 0.129). Ting RZ et al (2006)[12] observed clinically important and statistically significant association of vitamin B12 deficiency with duration of metformin use.

This study observed significant negative correlation coefficient (r=-0.65, p value<0.01) between dose of metformin and vitamin B12 levels. This observation was very consistent with the study of and Ting RZ et al (2006)[12] who also found significant inverse statistical association with the dose of metformin and vitamin B12 levels. Ting RZ et al (2006)[12] study demonstrated that with each 1gm/day metformin dose increment, it conferred an odd ratio of 2.88 (95% confidence interval 2.15-3.87) for developing vitamin B12 deficiency. Dose of metformin remained the strongest independent predictor of vitamin B12 deficiency. Ting RZ et al (2006)[12] study also explained similar results. Monique Nervo et al (2011)[30] showed association of dose of metformin with vitamin B12 levels as (r = -0.114, p = 0.173).

Although metformin therapy is associated with lower vitamin B12 levels, it does not appear to have any significant effect on peripheral neuropathy in those receiving metformin. This finding was consistent with the observations of Shihong Chen et al (2012)[7].

CONCLUSION
There was significant difference between the mean values of vitamin B12 levels in the metformin group and in the sulfonylurea and insulin groups (p value 0.0261 and 0.0751 respectively).

There was no statistical significant difference between the mean values of vitamin B12 in the diabetic population and the control from normal population (p value=0.6398).

There was significant difference between the mean values of vitamin B12, who has taken metformin for more than 1 year and in the mean values of vitamin B12, who has taken metformin for less than 1 year. P value was <0.0001 (significant).

The study observed significant negative correlation coefficient ®= -0.65, p value= <0.01) between dose of metformin and vitamin B12 levels.

From this study, it was concluded that vitamin B12 levels was definitely lowered with long term use of metformin.

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