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METFORMIN INDUCED VITAMIN B12 DEFICIENCY AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS.

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Abstract:

d**. Objectives:**
To study the effect of Metformin induced Vitamin B12 deficiency among patients with Type 2 diabetes mellitus (T2DM).

e. **Design:**
Case - control study.

f. **Setting:**
Outpatient clinics of Diabetic Endocrine Research Lab and patients reporting to medicine department of Sheth Vadilal Sarabhai General Hospital (Smt. NHL Municipal Medical College) , Ahmedabad, Gujarat .

g. **Participants:**
A total of 240 patients, 100 control group healthy normal and nondiabetic 140 study group with Type 2 Diabetes treated with Metformin and not known with a contraindication for the use of Metformin were approached, given informed consent, and entered the study.

h .**Intervention :**
Addition of Metformin to Insulin Therapy three times a day.

i. **Primary Outcomes :**
Vitamin B12, Folic acid ,Serum Homocysteine.

j. **Results :**
Compare with control, Metformin Treatment was associated with a mean difference in Vitamin B12 -340.26 and confidence 6.71 (95 % confidence interval 27.53 to 40.95 : P= < 0.0001) , and in Folate concentration of - 6.24 and confidence 0.18 (95% confidence interval 0.74 to 1.1: P=0.026) , and an increase in Homocysteine concentration of 18.97 and confidence 0.93 (95% confidence interval 3.83 to 5.69 : P= < 0.0001) . In addition, decrease Vitamin B12 and Folate could be explained by the increase in Homocysteine.

K. **Conclusion :**
Metformin increases the risk of Vitamin B12 deficiency and Folate, which results in a modest increase in Homocysteine concentration among patients with Type 2 Diabetes. Vitamin B12 Deficiency is preventable , therefore our findings suggests that regular measurements of Vitamin B12 during long term Metformin treatment should be strongly considered .

l. **Key Words:**
Diabetes, Homocysteine, Insulin, Metformin,Vitamin B12 and Folate.

Introduction:

Vitamin B12 or cobalamin is a water soluble vitamin that plays a very fundamental role in DNA synthesis and neurological function, hence an essential micronutrient required for Cardiovascular and Neuro-cognitive function[1]. Clinical Vitamin B12 deficiency and biochemical has been demonstrated to be highly prevalent among patients with Type 1 and Type 2 diabetes mellitus. Type 2 Diabetes Mellitus is associated with a two to four fold increase in the risk of cardiovascular disease[2], which cannot be fully explained by important risk factor such as hyperglycemia [3], hypertension [4] and dyslipidemia [5]. It also appears that Diabetes interact with risk factor to increase more risk of cardiovascular disease[6]. Hyperhomocysteinemia is a independent cardiovascular risk factor, specially in diabetic patient[7].For prevention of cardiovascular events in patients with Type 2 diabetes in addition for control of type 2 diabetes, identification and treatment of risk factor, such as hyperhomocysteinemia is critical[8]. It is well known that Metformin by decreasing plasma folate and Vitamin B12 levels can increase Serum homocysteine levels [9]. But data on this issues are sparse and conflicting [10,11,12].

In view of this consideration, the aim of the study was to evaluate the relation of Vitamin B12 and Folate and also to assess whether the high total homocysteine (tHcy) values had any say in the development of chronic complications like Nephropathy and Macroangiopathy of Type 2 diabetes in the setting of randomized, controlled trials.

Materials & Methods :

This study included total 240 patients,100 control group healthy patient and 140 study group with Type 2 diabetes Mellitus treated with Metformin , these patients have been referred to diabetic research laboratory and medicine department of Smt. N.H.L. Medical College , Ahmedabad , Gujarat . All patients gave informed voluntary consent to participate in the study. The study was approved by the ethic committee of Smt. N.H.L. Medical College.

We aimed to include the patient with Type 2 diabetes mellitus between 35 and 75 yrs of age during the study period from November 2011 to June 2016 and who had received a diagnosis of diabetes after 25 yrs of age, had never had an episode of keto-acidosis, and whose blood glucose lowering treatment had previously consisted of oral agents but now exclusive consist of insulin or insulin and Metformin. We excluded pregnant women and women trying to become pregnant and other serious medical or psychiatric disease.

Patients were randomized into 2 groups trial and control, Trial group treated initially with 850 mg Metformin daily. Doses of Metformin based on results of monthly Plasma glucose, as need be increased to 2000 mg daily .Whereas in control group 100 patients were non diabetic without any disease and healthy normal . Exclusion criteria were intolerance of Metformin or drugs which effect plasma homocysteine levels in duration of study.

Following Laboratory investigations were done in study and control group. Vitamin B12 by micro particle enzyme intrinsic factor assay, Folic acid by iron capture technology and glucose by Hexokinase, Serum homocysteine was determined by Fluorescence Polarization Immuno acid. All blood specimens were drawn at 0800 hrs after 12 hrs fast.

The normal level of Vitamin B12 are 208-963.5 pg/ml and folate is 7.2-15.4 ng/ml and below the normal range of this parameters is considered to be deficiency respectively [7,9]. The normal level of Serum Homocysteine are between 3.36–20.44 µmol/l. Serum homocysteine level of 20.44 µmol/l or more is considered to be hyperhomocysteinemia [13].

The data were analyzed using SPSS version 11 and P value less than 0.05 and 0.001 were considered to be statistically significant and highly significant respectively. Difference between mean Serum homocysteine levels in groups were assayed by student t- test and paired t- test. Pearson correlation test were applied to test correlation.

Result :

In final analysis , there were 240 Indian adults , ≥ 35 to 75 years of age with Type 2 Diabetes using Metformin (male 92 and female 48) , 100 adults normal healthy in same age group (

54 male and 46 female). The demography and biological characteristics of the groups are shown in Table 1. Among Metformin users mean age was 62.22 ± 6.47 years, 66% were male and 34% were female. The geometric mean serum vitamin B12 concentration among those with Type 2 Diabetes taking Metformin was 132.92pg/ml with standard deviation 29.39. This was significantly lower than the geometric mean concentration in those without diabetes(Control Group) 467.2 pg/ml with standard deviation 207.81, P=0.0116 . The prevalence of Biochemical Vit B 12 deficiency was 6.4% among those taking Metformin ≥ 1-3 years , 4.2% among those taking Metformin > 3-5 years , and 8.2% among those taking ≥ 5 – 10 years (P=0.3319 for ≥ 1-3 years vs ≥ 5 – 10 years . Similarly in patient with Metformin related folate deficiency, mean serum folate concentration was 5.56 ng/ml with standard deviation 1.71 as compared with 11.06 ng/ml standard deviation 2.64 in the control group. During Metformin treatment homocysteine increased, mean serum homocysteine 29.79 µmol/l with standard deviation 5.06 was compared with 12.02 µmol/l to 3.79 µmol/l in the control group. In the patient with Metformin in Type 2 Diabetes homocysteine level was compared in both male and female with control group. Serum homocysteine level was higher in male than female. That was corresponded by other studies (Alan et al., 2005: Passaro et al.2000)[14,15].

Table: 1 Socio demographics Profile / characteristics of study population analyzed .

	Metformin (n=140)	Control (n=100)
Demographics:		
Men : Women (n:n)	92:48	54:46
Age (years)	62.22(6.47)	50.75(11.46)
Currently Smoking n (%)	37(26)	19(19)
Duration of Diabetes Years	12.33(4.59)	NA
Insulin Treatment Years	8(9)	NA
Diabetic complications:		
Cardiovascular, n (%)	30(21)	NA
Retinal coagulation /Cataract extraction, n (%)	16(11)	NA
Amputation, n(%)	4(2)	NA
Metabolic variables:		
Body mass index (kg/m$^{2)}$	29(4)	29(5)
Weight (kg)	82(15)	84(14)
Systolic BP (mmHg)	122.06(15.78)	110.55(5.15)
Diastolic BP(mmHg)	77.28(8.14)	72.14(5.48)
Laboratory variables:		
Fasting plasma glucose(FBS)(mg/dl)	159.70(68.23)	90.81(13.01)
Post Prandial blood sugar(PP2BS) (mg/dl)	205.64(70.98)	100.33(12.54)
Vitamin B12 (pg/ml)	132.92(29.39)	467.16(207.81)
Folate (ng/ml)	5.56(1.71)	11.058(2.64)
Homocysteine (µmol/l)	29.79(5.06)	12.02(3.79)

*Values are in mean and standard deviation.

Compare with control, Metformin Treatment was associated with a mean difference in Vitamin B12 -334.24 and confidence 6.71 (95 % confidence interval 27.53 to 40.95 : P= < 0.0001) , and in Folate concentration of -5.50 and confidence 0.18 (95% confidence interval 0.74 to 1.1: P=0.026) , and an increase in Homocysteine concentration of 17.77 and confidence 0.93 (95% confidence interval 3.83 to 5.69 : P= < 0.0001) . In addition, the increase in Homocysteine could be explained by decrease in Folate and Vitamin B12.

Comparing the patients and controls, the levels of Serum Homocysteine were highly significantly higher among patients than controls, while vitamin B12 and folic acid level was found significantly decreased in patients than controls P (<0.0001) for each parameter. The effect of Metformin on the variables (serum homocysteine, folate and Vitamin B12) were re-analyzed with adjustment of age, previous Metformin treatment, duration of diabetes, gender , insulin dose and actual Metformin dose separately for each variable as well as all together, all of which did not materially change the result.

The our findings correlates and demonstrated as compare to published data (de Jager J 2010) that ,Metformin in Patients with Type 2 diabetes on long term (>5 yrs) lead to modest increase in Serum Homocysteine levels than patients exposed to short term (<5 yrs) and patients not exposed to Metformin: these changes were correlated . When we have calculated P value it was found to be statistically highly significant (p=<0.0001), and rate of significance is higher in study group than in control group.

The results and findings of our study correlates compared to published data (Wulffele at el, 2003) and which show that serum level of homocysteine in individuals with Type 2 Diabetes treated with It indicates that higher homocysteine level is observed in Type 2 Diabetes treated with Metformin which is a well known risk factor for the disease of the cardiovascular system which seem to be the main cause of increased mortality in patients with Type 2 Diabetes. Our study limitations are the smaller sample size over all and also we have a small number of female participants in comparison to males. This limitation may cause our inability to precisely evaluate completely the role of hyperhomocysteinemia in sex. Further study with more number of patients with more female participant is needed for more accuracy.

Conclusion:

In the present study, we have observed that Vitamin b12 folic acid deficiency and homocysteine level is significantly higher in patients of Type 2 Diabetes treated with Metformin compare to normal healthy subjects .Our findings are similar to those obtained by De Jager J et al , (2010) and Wulffele at el, (2003) that concluded in Patients with type 2 diabetes on long

term (>5 yrs) Metformin lead to modest increase in Serum Homocysteine levels than patients exposed to short term to Metformin and patients not exposed to Metformin: these changes were correlated .This means that Metformin therapy carries a potential risk for development of vitamin B12 and folic acid deficiency .

The risk of developing Metformin associated vitamin B12 deficiency is greatly influenced by increasing age, Metformin dose and duration of use .Our data favours that homocysteine level is affected by both non-modifiable risk factor of atherosclerosis like age and sex and modifiable risk factors like vitamin B12 and folic acid.

The study highlights the necessity of checking Vitamin B12 in patients with type 2 diabetes mellitus during Metformin treatment in order to avoid this potential adverse drug reaction and preserve the beneficial effects of Metformin.

Finally, from our study we can conclude that as Metformin is a cornerstone in the treatment of diabetes type 2, but make no recommendations on the detection and prevention of vitamin B12 deficiency during treatment. After completing the studies, we have an idea about the levels of these investigations such as Vitamin B12, folic acid Homocysteine, in patients with type 2 diabetes mellitus with Metformin, and our data provide a strong case for routine assessment of vitamin B-12 levels during treatment with Metformin.

So, person with increasing age (age above 35 years) should comprise Vitamin B12 in their health check-up and if there is any abnormality treat the underlying cause which will help individual to stay away from CVD in upcoming days up to some extent which seem to be the main cause of increased mortality in patients treated with Metformin in Type 2 Diabetes.

References:

1. Oh R, Brown D. Vitamin B12 deficiency. Am Fam Physician, 2003:67:979-86.(Pub med) (google Scholar)
2. Garcia MJ, McNamara PM. Gordon T.Kannel WB. Morbidity and Mortality in diabetes in the Framingham population. Sixteen years follow up study. *Diabetes* 1974; 23:105-11.
3. Stratton IM, Adler AI, Neil HA et al , association of Glycemia with macrovascular and microvascular complications of Type 2 diabetes (UKPDS35): prospective observational study . BMJ 2000; 321: 405-12.
4. UK prospective Diabetes study (UKPDS) Group , Tight Blood pressure control and risk of macrovascular and microvascular complications in Type 2 diabetes : UKPDS 38.BMJ1998; 317: 703-113.
5. Haffner SM. the Scandinavian Simvastatin Survival Study (4S) subgroup analysis of diabetic subjects: implications for the prevention of coronary heart disease [editorial; comment]. Diabetes Care 1997; 20:469-71.

6. Stamler J, Vaccaro O, Neaton JD, and Wentworth D (993).diabetes, other risk factors, and 12 year cardiovascular mortality for men screened in multiple risk factor intervention Trial. Diabetes care.16: 434-444.doi:10.2337/diacare.16.2.434.
7. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995).A Quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA. Doi: 10:1001/Jama .274.13.1049 274(13):1049-1057.
8. Wilson PW, Kannel WB, Sibershatz H, 1998, clustering of metabolic factors and coronary heart disease. Arch intern. Med. 159/10/1104/ 159(10):1104-1109.
9. Wuffele MG, kooy A , Lehert P, Bets D, Ogterop jc, Borger (2003). Effects of short –term treatment with Metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trail. J. int. Med. Doi: 10. 1046/j.1365-2796.2003.01213.x.254 (5):455-463.
10. Carlsen SM. Folling I. Grill V. Bjerve KS. Schneede J. Refsum H. Metformin increases total serum homocysteine levels in non –diabetic male patients with coronary heart disease. Scand J Clin Lab invest 1997:57:521-7.
11. Aarsand AK, Carlsen SM. Folate administration reduces circulating homocysteine levels in NIDDM patients on long-term Metformin treatment. J Intern Med 1998:244:169-74.
12. Hoogeveen EK. Kostense PJ. Jakobs C. Bouter LM. Heine RJ. Stehouwer CD. Does Metformin increase the serum total homocysteine level in non-insulin-dependent diabetes mellitus? J Intern Med 1997:242: 389-94.
13. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995; 274:1049-57.
14. Alan N, Elias, Steven Eng (2005). Homocysteine concentrations in patients with diabetes mellitus – relationship to microvascular and macrovascular disease. Diabetes Obes. Metab. Doi: 10.1111/j. 1463-1326.2004.00376.x.7 (2):117-121.
15. Passaro A, D' Elia K, Pareschi PL, Calzoni F , Carantoni M , Fellin R, Solini A (2000) . Factors influencing plasma homocyateine levels in Type 2 diabetes. Diabetes Care. Doi:10.2337/diacare.23.3.420.23 (3):420-421.