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RESEARCH ARTICLE

CLINICAL AND BIOCHEMIICAL MANIFESTATIONS OF VITAMIN B12 DEFICIENCY IN TYPE 2 DIABETIC PATIENTS TREATED WITH METFORMIN- A COMPARATIVE STUDY

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Abstract

Objectives: The objective of this study was to estimate the occurrence of Vitamin B12 deficiency in patients with type 2 diabetes mellitus treated with metformin, and the clinical and biochemical profile of vitamin B12 deficiency in them.

Materials and Methods: This was a cross sectional study. Type 2 Diabetics over the age of 18 years were recruited for the study. Patients were divided into two groups - one groupof 62 patients who were on metformin for more than 6 months and the other group of 62 patients who were on other oral hypoglycemic agents or insulin. Patients were interviewed using a questionnaire. Relevant blood investigations were done.

Results: The mean (+SD) age of study population was 57 (±9.8) years with an age range of 35 to 79 years. There was high prevalence of vitamin B12 deficiency in patients with type 2 diabetic mellitus treated with metformin (22.6%)(p-value<0.001). This study showed a statistically significant inverse relationship between duration of metformin use and vitamin B12 levels (p-value 0.01). There was a statistically significant correlation between the dose of metformin and vitamin B12 deficiency – the higher the dose, the lower the average vitamin B12 level (p-value 0.02). The study revealed significantly increased neuropathic symptoms in Type 2 Diabetic patients on metformin with B12 deficiency than with normal B12 levels (p-value<0.001).

Conclusions: This study demonstrated a high prevalence of vitamin B12 deficiency in patients with type 2 diabetic patients treated with metformin (22.6%). Hencethe measurement of vitamin B12 should become an essential part of the annual review in all patients with type 2 diabetic patients on metformin therapy. The study revealed significantly increased neuropathic symptoms in Type 2 Diabetic patients on metformin with B12 deficiency than with normal B12 levels.

Introduction:-

Diabetes Mellitus is one of the most prevalent chronic diseases, which is on the rise as a consequence of urbanisation and globalization [1]. India is amongst the top three countries with the highest burden of diabetic patients, with about 64.5 million adults with diabetes in the year 2014 as per a study published in the

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Lancet[2].Therefore Type 2 diabetes is currently a major public health problem. Type 2 diabetes mellitus results from impaired insulin secretion and reduced peripheral insulin sensitivity. Metformin, an oral biguanide is considered a cornerstone in the treatment of diabetes. It is a frequently prescribed first line therapy for individuals with type2 diabetes and reduceshyperglycemia by improving peripheral sensitivity to insulin. Biochemical and clinical vitamin B12 deficiency has been demonstrated to be common among patients with Type 2 diabetes mellitus[3]. Long-term and high-dose treatment with metformin is known to be associated with B12 deficiency[4][5]. Evidence from early clinical observation showed the prevalence of vitamin B12 malabsorption among patients undergoing long-term metformin treatment to be 30% [6]. The risk of adverse effects from metformin-induced vitamin B12 malabsorption was found to be increasing with the time of exposure to metformin.Depletion of vitamin B12 stores occurs after twelve to fifteen years of absolute vitamin B12 deficiency[7]. Therefore, in those patients who have been on long-term metformin, an annual vitamin B12 level should be obtained.

Methodology:-

It was a cross sectional study done by the department of General Medicine of St. John's hospital. Patients with Type 2 Diabetes Mellitus fulfilling the inclusion criteria were recruited for the study from the medical wards as well as on outpatient basis. Patients with diagnosed or previous history of other causes of B12 deficiency, pernicious anemia, pure vegetarians, post gastrectomy, malabsorption syndrome and those who were on B12 supplementation were all excluded from the study. The patients were divided into 2 groups -one group with 62 patients with Type 2 DM who were older than 18 years and were on metformin for more than six months and the other group with 62 patients with Type 2 Diabetes Mellitus who were on other oral hypoglycemic agents and insulin. After obtaining informed consent, the patients were interviewed using a questionnaire and detailed examination for signs of vitamin B12 deficiency including neurological examination was done. Relevant blood investigations such as Random Blood Sugar, complete hemogram, peripheral smear, mean corpuscular volume and vitamin B 12 levels were done. The prevalence of vitamin B12 deficiency were assessed in patients in metformin and non-metformin groups.The vitamin B12 deficiency value was taken as a cut of 220ng/L. Neuropathic assessment was done by Michigan Neuropathy Screening Instrument.

Institutional Ethics Committee (IEC), St. John's Medical College, Bangalore approved the conduct of the study. IEC Study Ref no. 338/2015.

Analysis

Data analysis was done using SPSS VERSION 20. Comparative analysis of numerical variables was done using t-test for parametric data and Mann-Whitney U test for non parametric data. Chi Square test was used for assessing association between categorical data. For all statistical testing $p < 0.05$ was considered significant.

Results:-

The mean(±SD) age under the metformin group was 57(±9.5) years as compared to 59(±10) years in the non metformin group. The mean duration of metformin use under the study was 9(±6.1) years with a median of 8 years with a range from 1year to 21 years.The mean metformin dose in metformin group under the study was 1345(±620) mg with a median value of 1500 mg. The values ranged from 500 to 2500 mg.

Table (i):- Mean Vitamin B12 values in Metformin and non – Metformin groups.

	Mean B12 value			
	Maximum (mg)	Minimum (mg)	Median (mg)	Mean (±SD) (mg)
Metformin group	2000	86	374.5	593.5(±515.5)
Non – metformin group	2000	247	832	957.8(±563.3)

It was found that14 out of 62 patients on metformin had Vitamin B12 deficiency whereas none of the 62 patients on other OHA or insulin had vitamin B12 deficiency. There was significant vitamin B12 deficency in the metformin group as compared to the non metformin group (p-value <0.001). The prevalence of vitamin B12 deficiency in patients on metformin was observed to be 22.6%.

Table (ii):- Metformin use and Vitamin B12 in patients on Metformin therapy.

	Duration of metformin use		Metformin dose		Vitamin B12 value	
	Median (range) (years)	Mean (±SD) (years)	Median (range) (mg)	Mean (± SD) (mg)	Median (range) (ng/L)	Mean (±SD)(ng/L)
B12 deficient group	17(1-21)	16(± 4.7)	2000 (500 – 2500)	2133(±516)	189 (86 – 212)	178(± 33)
B12 non – deficient group	6 (1-15)	6.6 (±4.6)	1000 (500 – 2000)	1093 (±420)	415 (250 – 2000)	725(±527)

Fig (i):- Duration of metformin vs Vitamin B12 levels.

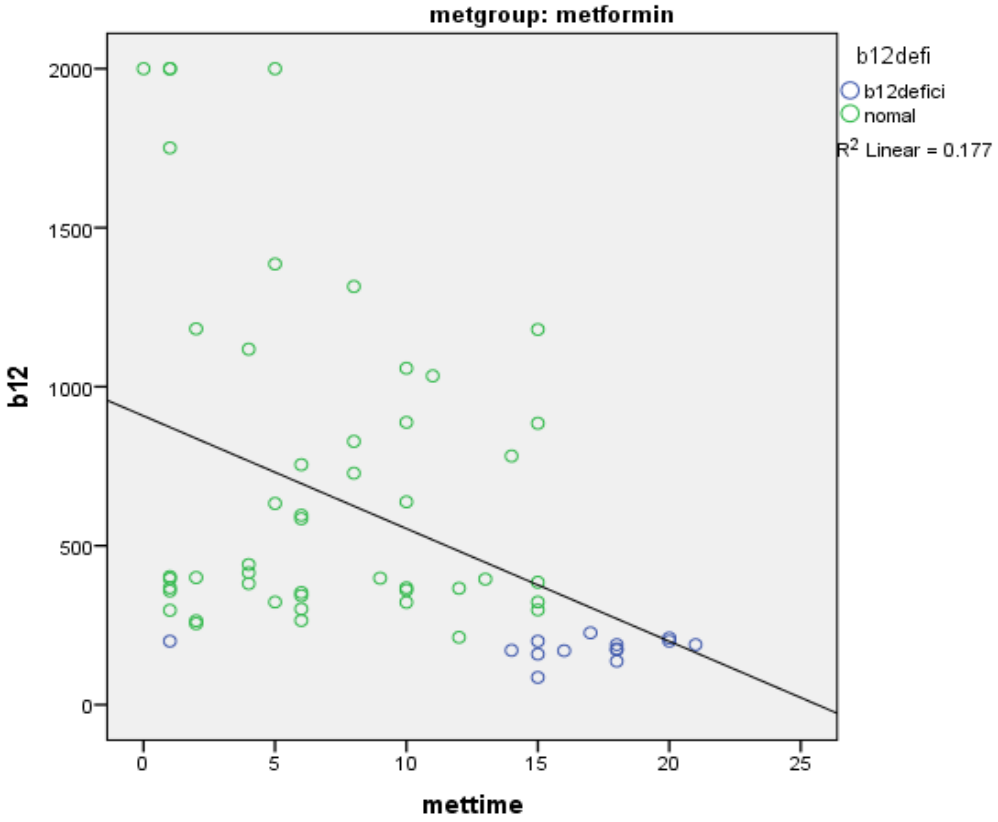
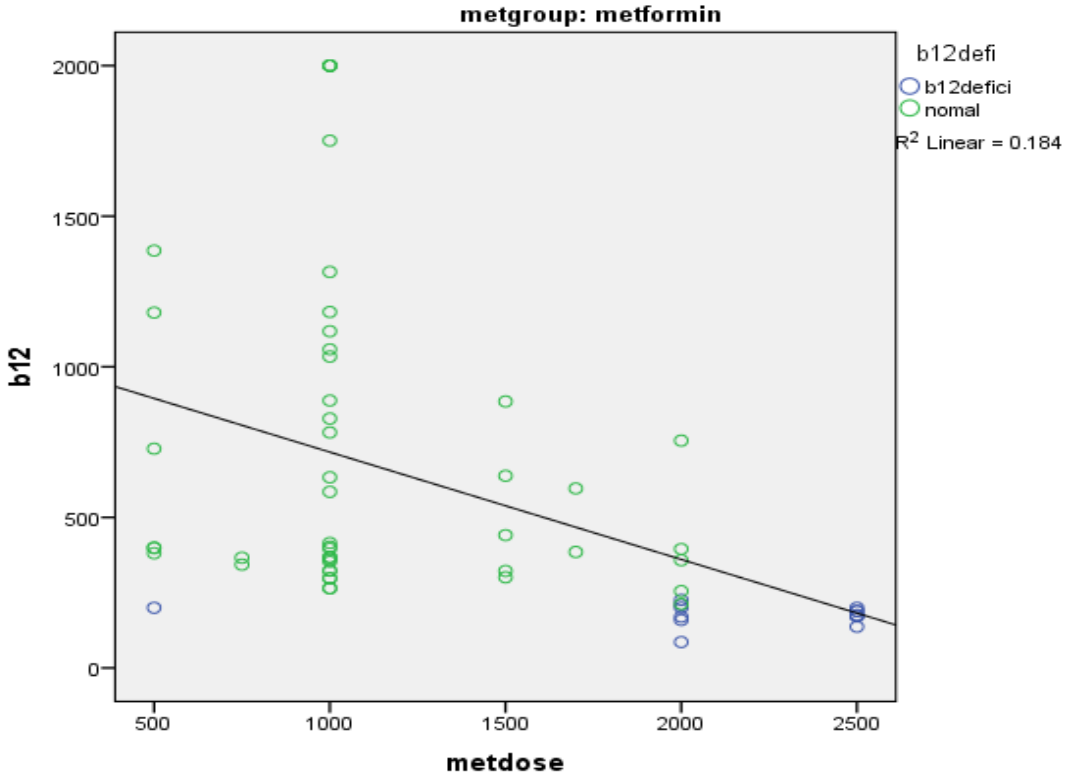


Fig (ii):- Dose of metformin vs Vitamin B12 levels(p-value-0.02)



Pvalue-0.0

Odds ratio calculation showed increase in chance of lowered serum Vitamin B12 level (<220 ng/L) with increase in dosage of metformin for more than 1000mg/day.

Table (iii):- Clinical manifestationin metformin group.

Variable		**Group based on metformin**		**Total (N)**	**P value**
		Non – Metformin (N)(%)	**Metformin (N)(%)**		
Pallor	No	50 (80.6%)	26(41.9)	61	< 0.001
	Yes	12(19.4%)	36(58.1)	63	
Mouth ulcer	No	62(51.20)	59(48.80)	121	0.242
	Yes	0(0)	3(100.00)	3	
Hyperpigmentation	No	62(51.70)	58(48.30)	120	0.127
	Yes	0(0)	4(100.00)	4	

Association of metformin intake with pallor was significant (p-value <0 .001).

Table (iv):- Neurological manifestations in metformin group.

Variable	Group	Group based on Metformin				Total		χ^2	df	P-value
		Non-Metformin		Metformin						
		N	%	N	%	N	%			
Vision impaired	No	62	51.20	59	48.80	121	100	1.366	1	.242
	Yes	0	0.00	3	100.00	3	100			
Dementia	No	62	50.40	61	49.60	123	100	.000	1	1.00
	Yes	0	0.00	1	100.00	1	100			
Tingling	No	50	58.80	35	41.20	85	100	8.416	1	.004
	Yes	12	30.80	27	69.20	39	100			

Glove stocking pattern	No	47	55.30	38	44.70	85	100	3.030	1	.082
	Yes	15	38.50	24	61.50	39	100			
Stabbing pain	No	47	58.00	34	42.00	81	100	6.017	1	.014
	Yes	15	34.90	28	65.10	43	100			
Numbness	No	47	59.50	32	40.50	79	100	7.848	1	.005
	Yes	15	33.30	30	66.70	45	100			
Pain sensory loss	Yes	12	40.00	18	60.00	30	100	1.583	1	.208
	No	50	53.20	44	46.80	94	100			
Touch sensory loss	Yes	12	38.70	19	61.30	31	100	2.108	1	.147
	No	50	53.80	43	46.20	93	100			
Temperature sensory loss	Yes	12	38.70	19	61.30	31	100	2.108	1	.147
	No	50	53.80	43	46.20	93	100			
Position sense impaired	Yes	8	66.70	14	33.30	12	100	1.476	1	.224
	No	54	48.20	48	51.80	112	100			
Vibration sense impaired	Yes	7	63.60	12	36.40	11	100	.898	1	.343
	No	55	48.70	50	51.30	113	100			

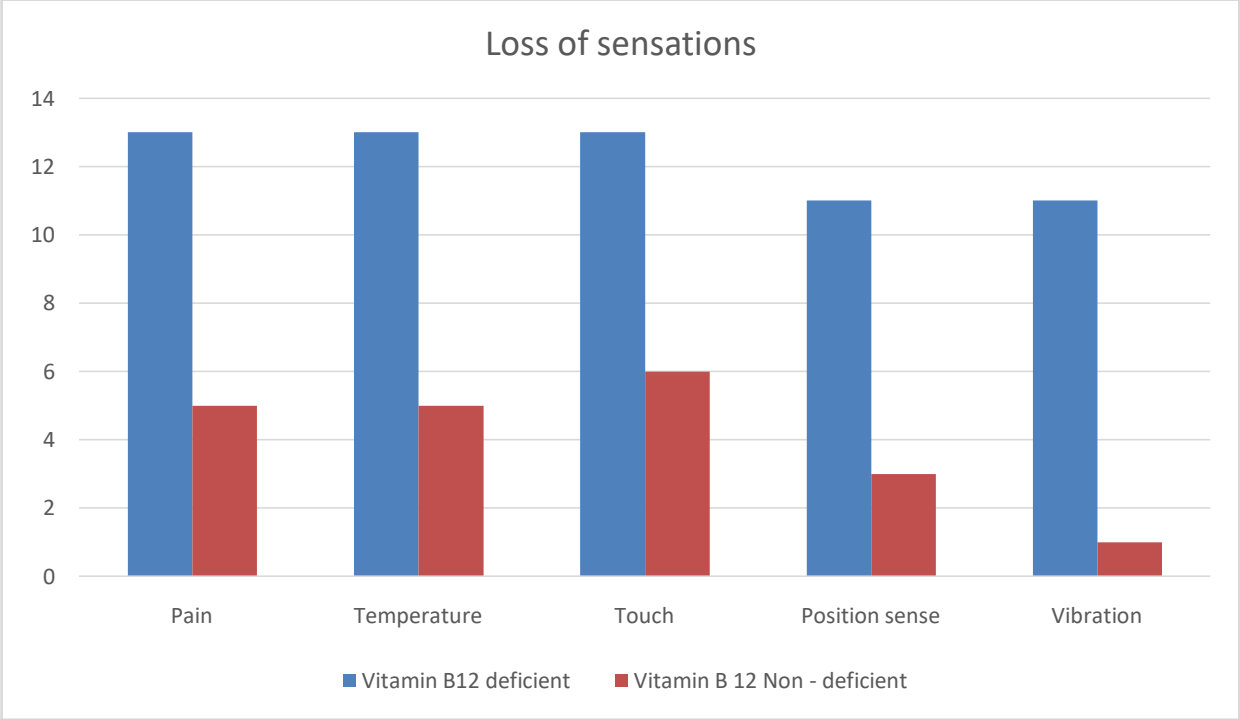
Tingling, stabbing pain and numbness were found to be related to metformin intake.(χ^2 (1) = 8.416, 7.848, p < .01, χ^2 (1) = 6.017, p < .05).

Table (v):-Neurological manifestation in Metformin group.

Variable	Group	Group -Metformin					p-value
		Non-Deficient		Deficient			
		N	%	N	%	N	
Tingling	No	34	70.80	1	7.10	35	.000
	Yes	14	29.20	13	92.90	27	
Glove sock	No	37	77.10	1	7.10	38	.000
	Yes	11	22.90	13	92.90	24	
Stab pain	No	33	68.80	1	7.10	34	.000
	Yes	15	31.20	13	92.90	28	
Numbness	No	31	64.60	1	7.10	32	.000
	Yes	17	35.40	13	92.90	30	
Pain sense loss	Yes	5	10.40	13	92.90	18	.000
	No	43	89.60	1	7.10	44	
Touch sense loss	Yes	6	12.50	13	92.90	19	.000
	No	42	87.50	1	7.10	43	
Tem sense loss	Yes	5	10.40	13	92.90	18	.000
	No	43	89.60	1	7.10	44	
Position sense impaired	Yes	3	6.20	11	78.60	14	.000
	No	45	93.80	3	21.40	48	
Vibration sense impaired	Yes	1	2.10	11	78.60	12	.000
	No	47	97.90	3	21.40	50	

All of the above were found to have a significant association with vitamin B12 deficiency.

Fig (iii):- Sensory loss and Vitamin B12 deficiency.



Discussion:-

In this study, the mean age under the group on metformin was 57(±9.5) years with a median age of 57 years whereas the mean age under the group non-metformin was 59(±10) years with a median age of 60 years. This observation was similar to the age of participants in an earlier study by Kang D et al[8].

It was evident from the study that metformin is a commonly prescribed therapeutic agent for glycemic control, hence putting the patients at risk of developing vitamin B12 deficiency. Patients who are seen in the OPD are often more complex, with associated comorbidities or diabetic complications, and often having poor glycemic control. This may lead to a higher level of vitamin B12 deficiency owing to factors such as poor diet and maintenance of health. However, these factors did not appear to have a significant influence on serum Vitamin B12 level. The overall level of prevalence that was observed for impaired vitamin B12 (serum vitaminB12<220 ng/L) of 22.6% of patients is similar to results from other studies with one study claiming a 40 % prevalence rate [9]. Pflipsen et al [3]studied that 22% of diabetic patients had metabolically confirmed vitamin B12 deficiency. Increasing age has been associated with lower Vitamin B12 status in some patients and may cause confounding interaction with some of the comparisons between the population groups. Our study showed statistically significant difference of B12 deficiency in the elderly age group (p-value 0.02) which was lower than those in the study by Green et al [9]. This comparison must be interpreted with caution as there are other factors that may affect the serum vitamin B12 of these patients which were not addressed in this study such as diet and drug interactions.

Duration of metformin treatment influences serum Vitamin B12 level. Due to the large natural stores of vitamin B12 in the human body of around 2500 µg and the reasonably low daily intake (2.4 µg) it is thought that a long period of malabsorption is required to cause clinically important Vitamin B12 deficiency[10]. The time point at which metformin use becomes a factor in the risk of vitamin B12 deficiency and some form of clinical presentation is commonly thought to be between 5 – 10 years of treatment [10]. However there have been observations made on the effect of metformin on vitamin B12 from as early as one year from commencement of treatment [11]. Our study found that patients using metformin for longer duration had statistically significant lower vitamin B12 levels(p-value 0.01). Several studies associate metformin use with established clinical vitamin B12 deficiency. In fact, higher doses and longer treatment with metformin seem to be risk factors for such deficiency[12]. Ting et al. showed an increased risk of vitamin B12 deficiency associated with longer duration and higher dose of metformin and found these parameters to be high risk factors for developing vitamin B12 deficiency[5]. The analysis of effect of dosage of

metformin on the serum vitamin B12 level has shown results supported by previous research[13] . An increase in the dosage of metformin correlates with an apparent decrease in serum vitamin B12. In one study, it was found that for every milligram increase in metformin dosage, serum vitamin B12 levels are lowered by 0.040 pmol/L[14]. In common dosage quantities this would suggest that a dosage difference of 1000 mg would result in an expected linear reduction of serum vitamin B12 by approximately 40 pmol/L.The association between increased dosage of metformin and decreased serum vitamin B12 level was observed in our study with a statistically significant p-value (0.02) . Through linear regression there was a significant decrease of serum vitamin B12 with increase in dosage; this can also be seen in a categorical dosage comparison. The reduction in serum vitamin B12 level translates to an increase in prevalence of decreased vitamin B12 status (through logistic regression).

Vitamin B12 deficiency can present with peripheral neuropathy. In this condition the transmission of nerve signals between the spinal cord and different parts of the body is disrupted. The problem may be due to a direct damage caused to the nerves or demyelination leading to axonal damage[15]. The study revealed significantly increased neuropathic symptoms with impaired posterior column sensationin Type 2 Diabetic patients on metformin with vitamin B12 deficiency than with normallevels (p- value<0.001). In the metformin group 30 (48%) out of 62 had neuropathy and in non metformin group 15(15%) out of 62 had neuropathy, and in the vitamin B12-deficient patients on metformin, 13 of 14 (93%) had neuropathy compared to six out of 48 (12%) in group on metformin with normal vitamin B12 levels. Thisis comparable with the study in Royal College of Surgeons in Ireland Student Medical Journal[16].In the vitamin B12-deficient patients on metformin, 4 out of 53 (7.5%) recorded values indicated neuropathy compared to 6 out of 108 (4%) recorded values for the patients on metformin with normal vitamin B12 levels. Among the vitamin B12-deficient control (5 patients), 3 neuropathy values were recorded, 1 of which was positive (33%). Within the normal controls, 42 neuropathy values were recorded, one of which was positive (2%). Another study showedmean serum vitamin B12 levels were significantly lower in metformin exposed group compared with non-metformin exposed group, with a significantly highermean neuropathy in metformin exposed group.

Conclusions:-

This study demonstrated a high prevalence of vitamin B12 deficiency in patients with T2DM treated with metformin(23%). Hence suggesting that the measurement of vitamin B12 should become an essential part of the annual review in all patients with T2DM on metformin therapy. In this study the data showed a statistically significant inverse relationship between duration of metformin use and vitamin B12 levels. There was a statistically significant correlation between the dose of metformin and vitamin B12 deficiency – the higher the dose, the lower the average vitamin B12 level. The study revealed significantly increased neuropathic symptoms with impaired posterior column sensationin Type 2 Diabetic patients on metformin with B12 deficiency than with normal vitamin B12 levels. The study also revealed that there was statistically significant correlation of vitamin B12 deficiency among the group aged above 60 years.

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