METFORMIN THERAPY: A COMPARATIVE STUDY OF VITAMIN B$_{12}$ DEFICIENCY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Diabetes Mellitus is one of the most common endocrine disorder whereas metformin is the drug of choice and frequently used hypoglycemic agent. Metformin side effects are ignored by most of the physician, although it causes vitamin B$_{12}$ defeciency.

Methods: This cross-sectional analytical study was carried out to determine the frequency of Vitamin B$_{12}$ deficiency in Type 2 Diabetes Mellitus patients taking metformin compared with those not taking metformin, in a tertiary care hospital (Hayatabad Medical Complex) Peshawar from July to December 2015. The study population comprised of patients with Type 2 Diabetes Mellitus divided into two groups (group1 using metformin and group 2 not using metformin therapy). A total of 110 patients of both gender were recruited in the study. Data were collected on a structured performa and analyzed using SPSS version 20. Students "t" test and Chi-square test were used for the comparison of proportion among two study groups, where statistical significance was accepted at $P \leq 0.05$

Results: Total 139 patients were requested for participation out of which 110 responded, 54 male and 56 female patients. Age ranged between 35 to75 years, mean age was 55.42 ± 9.6 years. The results revealed that, 6 (5.5%) of the total study population had vitamin-B$_{12}$ deficiency out of whome all were from group-1 (those using metformin).45 (40.9%) patients had indeterminate deficiency but the difference among the two groups was not significant.

Conclusion: The study showed that Type 2 Diabetic patients using metformin had vitamin B$_{12}$ deficiency and related complications. So,Type-2 Diabetes Mellitus patients on long term metformin should be screened for Vitamin B$_{12}$ deficiency and if found deficient should be treated.

INTRODUCTION

Vitamin B$_{12}$ (Cyanocobalamin) is a water soluble vitamin which plays an important role in haemopoesis, DNA synthesis as well as neurological functions.[1]The absorption of vitamin B$_{12}$ is associated with the formation of vitamin B$_{12}$ complex with intrinsic factor (IF) which is secreted by specialized parietal cells of gastric mucosa. This vitamin B$_{12}$–IF complex is capable to resist the action of proteolytic enzymes and prevent its degradation. However metformin, a first choice of oral hypoglycemic agent in T2DM (Type 2 Diabetes Mellitus) cause vitamin B$_{-12}$malabsorption, which in turn may lead to vitamin B$_{12}$ deficiency and complications linked with its deficiency [2,3]. About 10-30% patients with Diabetes Mellitus who are on metformin had suffered from vitamin B$_{12}$ deficiency. [4,5] The clinically negative consequences of metformin are increased in plasma folate deficiency along with vitamin B$_{12}$defeciency [6].

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In T2DM metformin is one of the most commonly used oral hypoglycemic drug in the world, and has been recommended by most of the well reputed diabetic associations including "American Diabetes Association", "Korean Diabetes Association", and "European Association for the Study of Diabetes" [7]

Jolien de Jager et al [3] studied that there was a mean decrease of vitamin B$_{12}$ concentration in 19% patients on long term metformin therapy for more than 4years which is preventable.

According to Bauman et al.[5],10-30% patients who were on metformin therapy for a long time had vitamin B$_{12}$ deficiency as a result of malabsorption due to antagonist effect of metformin on calcium dependent vitamin B$_{12}$-intrinsic factor complex uptake by ileal cell surface receptors. Similarly study conducted by Kang D et al.[8], 22% of patients with T2DM had vitamin B$_{12}$ deficiency due to its metabolic alteration.Another study conducted by Ting et al.[2],reveals that 50% of patients have metformin related vitamin B$_{12}$ deficiency, most importantly those patients using metformin for more than 3yrs were at higher risk of developing vitamin B$_{12}$ deficiency. Results from a comparative study by Filioussi K et al[9] revealed that 40% of study population on metformin treatment suffered from macrocytic anemia as compared to control(non-metformin group) due to reduced serum vitamin B$_{12}$ level. Few studies in Khyber Pakhtunkhwa Pakistan have been conducted to see the

frequency or prevalence of vitamin B_{12} deficiency among type 2 diabetic patients on metformin therapy.

MATERIAL AND METHODS

This cross-sectional analytical study was carried out to determine the frequency and compare vitamin B_{12} deficiency in patients with T2DM on metformin therapy and without metformin use.This study was conducted in Medical and endocrinology departments of Hayatabad Medical Complex Peshawar. Lab procedures were performed in pathology lab at Rehman Medical Institute Peshawar and completed in a period of six months. Both male and female patients with T2DM who satisfy the inclusion and exclusion criteria were enrolled. Convenient sampling technique was used and patients divided in two groups as

a) Patients of T2DM who were on metformin therapy (n=56)

b) Known cases of T2DM with no history of metformin therapy (n=54)

The data were collected on structure Performa. Detailed history was taken and thorough clinical examination done. While following selection of subjects, informed consent was taken about the proposed study. 5ml of venous blood was obtained by veni-puncture under aseptic precautions. Blood for smear, glucose measurement and HbA1c was transported to lab in EDTA bottle while blood for serum was taken in non-EDTA bottle.

Regarding biochemical profile (serum vitamin B_{12}), blood sample collected was then centrifuged; the serum obtained was stored at −8°C until the analysis. Serum vitamin B_{12} concentration was measured by using ECLIA "Electrochemiluminescence assay". Serum vitaminB_{12} levels <200 pg/mL were considered deficient whereasvitamin B_{12} levels more than 400pg/mL were considered normal. Blood levels of B_{12} between 200 and 400pg/mL were considered indeterminate.[10,11]

Data analysis was done using SPSS version 20 and MS excel. Statistical significance of difference between two groups was determined by using Students "t" test and Chi-square test , where statistical significance was accepted at $P \leq 0.05$

RESULTS

A total of 110 out of 139 sampled subjects (response rate 79.1%) with mean age 55.42±9.6 (ranged from 35- 75 years) participated in the study, of whom 56 (50.9%) were the cases (T2DM on metformin) and 54 (49.1%) were Control (T2DM without metformin) (Fig 1). Among total 56(50.9%) were female and 54(49.1%) were male (Fig 2). Further the age categories indicates that majority 40(36.4%) of them were from 45 - 55 Years group followed 35(31.8%) from 55 - 65 Years, 19(17.3%) from 35 - 45 Years and 16(14.5%) from 65 - 75 Years
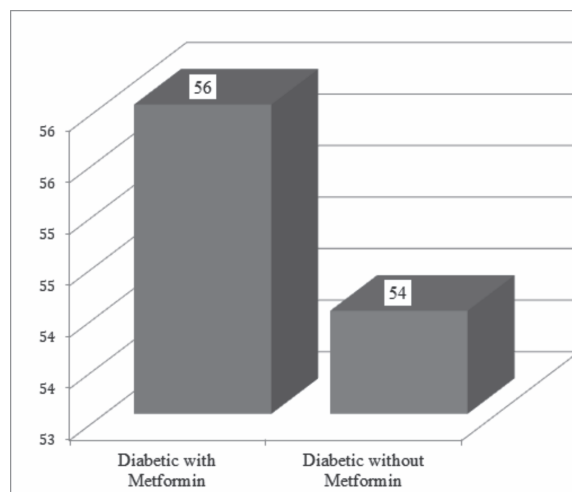

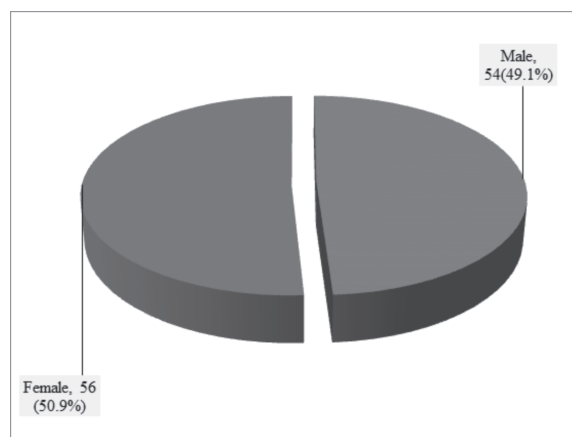Figure 1: Distributions of study participants by cases and control

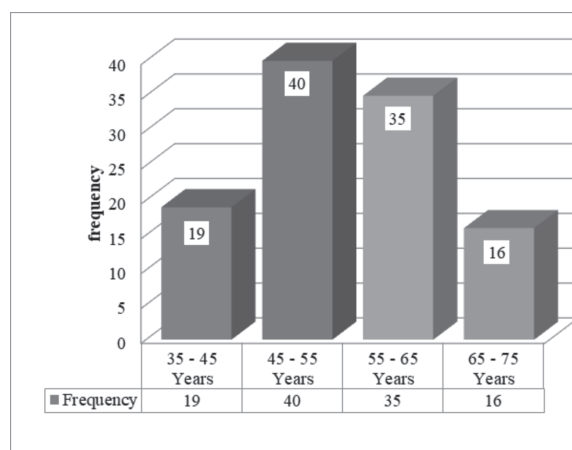Figure 2: Distribution of the participant with respect to their gender

Figure 3: distribution of the Participants with respect to their Age categories

Table 1: Prevalence of Vitamin-B12 Deficiency

Prevalence of Vit-B$_{12}$ defeciency	Therapy		Total	P-Value
	Metformin	Non metformin		
Deficient in Vit-B12	6 (5.5%)	0 (0.0%)	6 (5.5%)	0.047
Indeterminate Deficiency	45 (40.9%)	49 (44.5%)	94 (85.5%)	
Normal	5 (4.5%)	5 (4.5%)	10 (9.1%)	
Count % of Total	56 (50.9%)	54 (49.1%)	110 (100%)	

Table 2: Comparison of Biochemical and Hematological indices in patients with and without metformin therapy

Parameter	Mean ± SD Diabetic with Metformin	Mean ±SD Diabetic without Metformin	Mean differences	95% Confidence Interval of the Difference		P-Value
				Lower	Upper	
RBS	196.64±58.6	197.54±52.843	-0.894	-22.0	20.2	0.93
Hb	12.4±1.9	13.1±1.8	0.75	-1.4	-0.055	0.035
TLC	9135.7±3738.0	8711.4±2772.1	424.2	-823.1	1671.6	0.50
Platelets	329250.0±128930.0	340888.8±145912.9	-11638.8	-63630.5	40352.7	0.65
MCV	84.1±8.3	81.8±8.7	-2.2	-5.5	0.96	0.16
Vit-B12	195.10±131.5	270±256.3	-75.0	-153.0	2.5	0.05
HbA1c	9.3±3.0	10.2±2.4	-0.88	-1.9	0.1	0.09

age group. (Fig 3)

Vitamin B$_{12}$ status among the study population

The results revealed when both groups, Type 2 Diabetes Mellitus on metformin and Type 2 Diabetes Mellitus patients not using metformin, 6 (5.5%) of the total study population had vit-B$_{12}$ deficiency out of whome all were from group-1 (those using metformin). 45 (40.9%) patients had indeterminate deficiency but the difference among the two groups was not significant. Normal range of vitamin B$_{12}$ reported only among 5 (4.5%) patients on metformin use and 5 (4.5%) patients non-metformin users. Table 1

Comparison of Hematological determinants in T2DM patients with and without metformin use.

The hematological indicators like; Hb, TLC, Platelets count, MCV were investigated during this study. The main variables of interest was Vitamin B$_{12}$ and HbA1c were also compared in both of the groups (Diabetic patients with and without Metformin)

DISCUSSION

Metformin is one of the most widely used drug for treatment of T2DM in the world. Long term metformin therapy results in vitamin B$_{12}$ deficiency among 30% of T2DM patients[9]

This cross-sectional analytical study in KP,Pakistan which was designed to determine and com-parevitamin B$_{12}$ deficiency in adults with T2DM taking metformin with those not taking metformin.

Results of present study indicated that 6(5.5%) of the total study population (n=110) had Vitamin-B$_{12}$ deficiency out of whome all were from group-1 (those using metformin). Similarly 45 patients (40.9%) had indeterminate deficiency but the difference among the two groups was not significant. The results of the present study is consistent with Matthew C et.al.[12] According to them few patients suffered from vitamin-B$_{12}$ deficiency and 54% of diabetic patients with metformin had indeterminate deficiency i.e. Vitamin B$_{12}$ level ranged between 200 and 400pg/mL. A study by Reintatatler L[13], revealed that the prevalence of vit-B$_{12}$ deficiency was 5.8% among T2DM patients who were on metformin therapy as compare 2.4% among those T2DM patient who were not using metformin (P = 0.0026)[13]. These results consistent with and support the findings of the present study. The metformin therapy has been reported to decrease serum vitaminB$_{12}$ level as indicated in efficacy trial conducted by DeFronzo and Goodman[14].

The comparative analysis of vitamin B$_{12}$ level in the present study indicates mean Vitamin-B$_{12}$ level in patients on metformin therapy was 195.10±131.5 pg/mL as compare to 270±256.3pg/ml in patients who were not using metformin, which accounted for a mean difference of 75.0 pg/ml,which is consistence with study byTing RZ,[2] and De deger J, [3] which also indicates between 10% -30% of people who take metformin on

regular basis have evidence of decreased vitaminB$_{12}$ absorption. Vitamin B$_{12}$ level was significantly lower in patients who have been on metformin for ≥10 years compared with those patients with <10 years history of metformin use. Similarly, patients who were on metformin at a dose of >1000 mg/day had significantly lower vitamin B$_{12}$ level when compared with patients on ≤1000 mg/day.[15] Similarly RaheelIftikhar R, et al,[16] also reported that 31% of patients with metformin had vitamin B$_{12}$defeciencyas compared to 8.6% among control (p=0.002). Dose of metformin had inverse correlation with vitaminB$_{12}$ level and the difference was statistically significant (p<0.001)[16] Similar studies from other countries (Brazil) by Ghazanfari Z,[17]also show low vitamin B$_{12}$ level in 6.9% of patients on metformin.

CONCLUSION

From the study results and respective literature review it is concluded that metformin use and its dose was positively associated with Vitamin B$_{12}$ deficiency. Thus it is recommended that vitamin B$_{12}$ and routine hematological profile screening of patients with T2DM should be adopted in practice which may help to prevent complications associated with Vitamin B$_{12}$ deficiency and if found deficient shall be treated.

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