Original Article

Vitamin Cobalamin in Metformin Treated Type 2 Diabetics Presenting at a Tertiary Care Hospital of Sindh

Muhammad Akbar[1], Akram Munir[2] and Fatima Qureshi[2]

ABSTRACT

Objective: To determine vitamin cobalamin (VC) in metformin treated type 2 diabetics compared to no metformin treated type 2 diabetics.

Study Design: Observational study

Place and Duration of Study: This study was conducted at the Isra University Hospital, from March 2019 to February 2020.

Materials and Methods: A sample of 221 diabetics taking metformin (Group A- cases) and 200 diabetics (no metformin) (Group B - control) were selected through non-probability convenient sampling. Blood was centrifuged to get sera for analysis. Cobalamin was detected by ELISA assay kit (Chemiluminescence Technique). Data was analyzed and tabulated on SPSS (version 21.0) at 95% CI.

Results: Mean VC levels in group A (diabetics taking metformin) and group B (diabetic not taking metformin) were found at 139.5±15.7 and 302.5±58.51 pg/dl respectively (P=0.0001). VC deficiency was found in 83.3% in diabetics taking metformin compared to 71.5% in diabetic not taking metformin.

Conclusion: We found cobalamin deficiency in 83.3% diabetics taking metformin compared to 71.5% diabetic not taking metformin.

Key Words: Cobalamin, Metformin, Type 2 Diabetics.

Citation of article: Akbar M, Munir A, Qureshi F. Vitamin Cobalamin in Metformin Treated Type 2 Diabetics Presenting at a Tertiary Care Hospital of Sindh. Med Forum 2020;31(10):194-198.

INTRODUCTION

World burden of type 2 diabetes mellitus was 463 million diabetics in 2019 that is estimated to rise 700 million by the year 2045 as reported by the International Diabetes Federation (IDF).[1] Second National Diabetes Survey of Pakistan (NDSP) 2016 – 2017 showed the DM prevalence was 26.3%.[1,2] Metformin is the first line of hypoglycemic drugs recommended by the American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD) and the IDF.[1,3] Previous studies had revealed metformin therapy is associated with vitamin cobalamin (VC) deficiency.[4] Previous studies[4-6] reported VC deficiency occurrence in 9% to 52% of type 2 diabetics and metformin has been an alleged drug. VC is necessary for the re-methylation reaction of homocysteine (Hcy) to methionine. VC is essential for this methylation reaction of Hcy and VC deficiency may cause hyperhomocysteinemia – that is a risk factor for the vascular complications in type 2 diabetics.[7] Vitamin cobalamin insufficiency or deficiency has emerged as an epidemic in metformin treated diabetics. Long term intake of metformin is reportedly interferes with intestinal cobalamin absorption. Cobalamin deficiency is associated with hematological disorders, immune dysfunction, hyperhomocyteinemia, vascular and neurological complications.[8] Metformin induced cobalamin deficiency has been categorized as minor (type B) drug induced condition of clinical significance. Metformin when taken chronically for average >5 years manifests low serum cobalamin levels. Deficiency occurs for >5years duration till hepatic deposits is depleted. Metformin belongs to the biguanide class of anti – diabetic drug widely used because of low cost.[8,9] Metformin augments insulin sensitivity and inhibits hepatic gluco-neogenesis.[10] It has very good safety profile having gut intolerance as the major complaint.[11] In Pakistan, prevalence of DM has exponentially increased[2,12] compared to any other country affecting rural and urban populations equally. Metformin, being cost effective drug, is widely used in the country. Risk of VC deficiency in long term metformin intake diabetics needs further studies as the risk of VC deficiency is multiplied by dietary insufficiency.[13] In this context the present observational study was conducted to analyze the serum cobalamin in type 2

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Received: April, 2020
Accepted: July, 2020
Printed: October, 2020

diabetics taking metformin for long durations presenting at our tertiary care hospital.

MATERIALS AND METHODS

The present observational study was conducted at the Department of Medicine, Isra University Hospital. Study took place from March 2019 to February 2020. Study was approved by the institute`s ethical review committee (ERC). A sample of 221 diabetics taking metformin (Group A- cases) and 200 diabetics (no metformin) (Group B - control) were selected through non-probability convenient sampling. Sample size was calculated by proportions for the sample size. Inclusion criteria for group A were; diagnosed cases of DM, volunteers, age 40 – 60 years, metformin intake for ≥5 years and metformin dose 1-2 grams daily. Inclusion criteria for group B were; diagnosed cases of DM, volunteers, age 40 – 60 years, who had no history of metformin intake. Exclusion criteria were; age <40 or > 60 years, meat intake, liver or liver extract intake in last 3 months, hypothyroid, alcoholics, chronic liver disorders, renal disease, chronic malabsorption syndrome and taking proton pump inhibitors. Patients taking multivitamins and vitamin-mineral pills were excluded. Signing of consent form was mandatory. Duration of diabetes mellitus, metformin intake, relevant clinical history, and other drug intake was taken. Clinical history was taken by researcher to fulfill the inclusion and exclusion criteria. Volunteers were interviewed of their full consent, signing of proforma and willingness of blood sampling. They were asked to abide by the study protocol study. They were informed that the expenses of blood investigations will be paid by the researcher. Volunteers were informed the confidentiality of information is secured by the researcher, and blood laboratory investigations will never by publicized, only results will be published in a medical journal. A pre – structured proforma was designed for data collection to be entered and saved in. Volunteers were taken 3 ml blood from skin vein, preferably from the ante – cubital fossa under proper aseptic conditions. 3 ml BD disposable syringe drew blood; 3 ml was put into Sodium fluoride tube, and 2 ml was centrifuged to get sera. Hematolgoical parameters were performed in Cobas hematology analyzer. Sera were used for vitamin cobalamin (VC) estimation by ELISA assay kit (Abbot Architect -1000) by Chemiluminescence Technique. Cobalamin levels were categorized as; <100 pg/ml - severe VC deficiency, <170 - >100 pg/dl VC deficiency, 170-239.9 pg/ml – mild deficiency and ≥240pg/ml normal vitamin cobalamin levels.[14] Statistical analysis of variables was performed on SPSS (version 21.0) unisng Student t – test and results were presented as mean and standard deviation (SD). Gender distribution was analyzed by Chi-square and results (frequency and %). Results were taken statistically significant at 95% CI ($P \leq 0.05$).

RESULTS

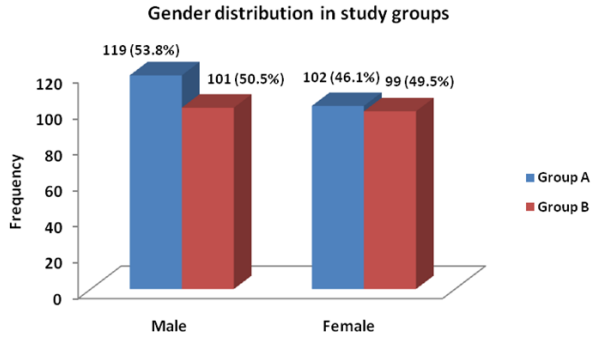
Mean age of subjects in Group A (Diabetics) was 48.5±7.5 compared to Group B (control) 47.5±11.5 years. Group A comprised of 221 study subjects; 119 (53.8%) were male and 102 (46.1%) were female (P<0.05). While Group B comprised 200 study subjects; male were 101(50.5%) and female were 99 (49.5%)(P>0.05). Mean vitamin cobalamin levels in group A (diabetics taking metformin) and group B (diabetic not taking metformin) were found at 139.5±15.7 and 302.5±58.51 pg/dl respectively (P=0.0001).

Table No.1: Cobalamin levels in study groups

Cobalamin	Group A. Diabetics (n=221)	Group B. Control (n=200)	P
<100 pg/dl	70.5±15.1	97.1±2.9	0.0001
<170 - >100 pg/dl	111.1±5.6	165.2±37.5	
170-239.9 pg/dl	191.3±11.5	276.3±34.3	
≥240 pg/ml	615.1±13.7	935.7±26.7	

Table No.2: Frequency of cobalamin deficiency in study groups

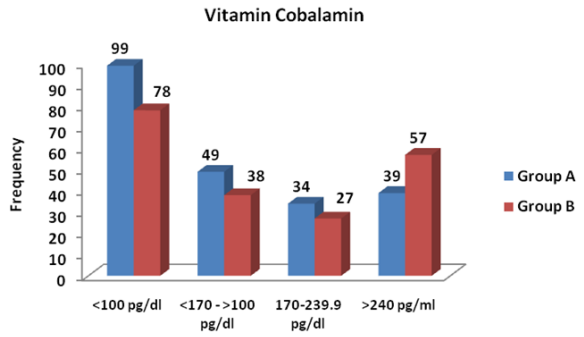
Cobalamin	Group A. Diabetics (n=221)	Group B. Control (n=200)	P
<100 pg/dl	99 (44.7%)	78 (39%)	0.0001
<170 - >100 pg/dl	49 (22.1%)	38 (19%)	
170-239.9 pg/dl	34 (17%)	27 (13.5%)	
≥240 pg/ml	39 (15.3%)	57 (28.5%)	
Total	221 (100%)	200 (100%)	



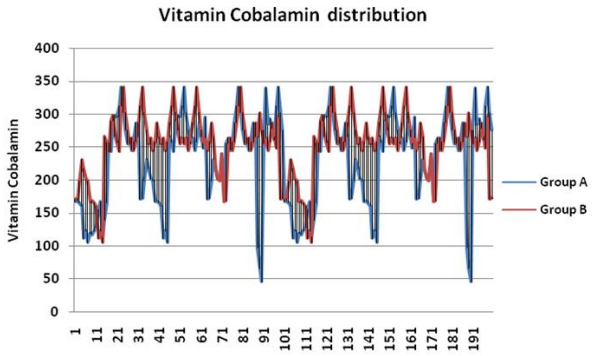
Graph No.1: Gender distribution in study groups

Table – 1 shows the vitamin cobalamin distribution in study groups. In group A, vitamin Cobalamin levels; severe deficiency (<100 pg/dl), mild deficiency (170-240 pg/dl), deficiency (<170 pg/dl) and normal (>240 pg/ml) levels were found in 49.5%, 24.5%, 17% and

19.5% compared to group B that revealed severe deficiency (<100 pg/dl), mild deficiency (170-240 pg/dl), deficiency (<170 pg/dl) and normal (≥240 pg/ml) levels in 39%, 19%, 13.5% and 28.5% respectively (table 2 and graph 2) (P=0.0001). Graph 3 shows the vitamin Cobalamin distribution in groups A and B. Normal and deficient cobalamin was found in 16.2% and 83.3% in group A (diabetics taking metformin) compared to 28.5% and 71.5% in group B (diabetic not taking metformin).



Graph No.2: Frequency of vitamin cobalamin levels distribution



Graph No.3: Vitamin cobalamin levels in group A and B

DISCUSSION

The present observational study was conducted at our tertiary hospital Liaquat University, Jamshoro. Serum cobalamin levels were comapred between diabetics taking metformin versus diabetics not taking metformin. Metformin is a cost effective drug hence prescribed as first line drug for glycemic control in type 2 diabetics. It is prescribed as single pill or combined with other anti – diabetic drug. Gut intolerance is the common side effect of metformin; however, long term therapy in diabetics has been implicated in causing vitamin cobalamin (VC) deficiency. One postulated mechanism of VC deficiency is retardation of cobalamin-intrinsic factor endocytosis in the terminal ileum. In present study, cobalamin deficiency was found in 83.3% group A (diabetics taking metformin) compared to 71.5% in group B (diabetic not taking metformin). The findings are in agreement with previous studies.[1,15,16] A recent study[15] from Pakistan reported 68% VC deficiency in gestational DM compared to 40% in control. They reported that the vitamin cobalamin deficiency is very common nutritional deficiency. Another recent study[1] from Pakistan has reported the VC insufficiency is frequent findings in metformin treated type 2 diabetics that may progress to frank VC deficiency. They found association of VC deficiency causing peripheral neuropathy. The findings of above studies are in agreement with present study. However, 83.3% group A (diabetics taking metformin) is a new finding showing the true prevalence of metformin – induced VC deficiency. Still another recent study[16] has reported 41% VC deficiency in metformin treated type 2 diabetics compared to 20% in metformin naïve patients. The findings of above study are in keeping with present study finding of VC deficiency. However, frequency of 83.3% cobalamin deficiency is very high and is being reported for the first time. This may be because of nutritional deficiency that is prevalent among the general population of Pakistan.[17-19] We think the nutritional VC deficiency is very high in the society and metformin – induced addition shows high VC deficiency. This is the most probable justification of VC deficiency. Another justification of high VC deficiency is the 71.5% VC deficiency in no-metformin diabetics that points to the severe nutritional deficiency.[17-19] A previous study[20] reported 27.5% VC deficiency in metformin treated diabetics. The finding of VC deficiency is a consistent finding but the high frequency of 83.3% was found in present study. Another reason of severe VC deficiency is duration of metformin as the present study selected those diabetics with history of ≥ 5 years metformin therapy. Nutritional deficiency is more prevalent and this may be superadded by the metformin therapy causing severe VC deficiency.[17-19] A previous study[21] reported 31% VC deficiency in metformin treated diabetics that is in contrast to 83.3% VC deficiency in present study. This controversy is because of duration of metformin therapy. Above study analyzed VC at 3 months that is in contrast to ≥5years metformin duration of present study. Another previous study[22] found 31% VC deficiency in metformin treated diabetics compared to 9% no- metformin therapy. In present study, cobalamin deficiency was found in 83.3% diabetics taking metformin compared to 71.5% diabetic not taking metformin. This indicates prevalent nutritional deficiency in the country. A previous study[23] have reported 38.1% VC deficiency in their diabetic population. However, the study sample of above study was small. Hence findings are incomparable. In present study, the mean vitamin cobalamin levels in group A (diabetics taking metformin) and group B (diabetic not taking metformin) were found at 139.5±15.7 and

302.5±58.51 pg/dl respectively (P=0.0001). The findings are in agreement with a previous study[24] that reported VC deficiency in range of 125–250 pg/ml. A previous study[25] found VC deficiency that was severe enough causing megaloblastic anemia in metformin treated diabetics. The findings are sufficiently keeping with the present study. A previous study showed VC deficiency <150 pmol/l that is in agreement as the present study found <100 pg/dl VC concentration in both study groups. One of the major limitations of present study is that the nutritional deficiency might have affected the results towards severe VC deficiency that needs further studies.

CONCLUSION

Vitamin cobalamin deficiency is observed in diabetics taking metformin therapy for long duration. We found cobalamin deficiency in 83.3% diabetics taking metformin compared to 71.5% diabetic not taking metformin. Diabetics taking metformin for long duration must be checked vitamin cobalamin and should get vitamin supplements.

Author's Contribution:

Concept & Design of Study:	Muhammad Akbar
Drafting:	Akram Munir
Data Analysis:	Fatima Qureshi
Revisiting Critically:	Muhammad Akbar, Akram Munir
Final Approval of version:	Muhammad Akbar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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