

**SERUM HOMOCYSTEINE AND VITAMIN B₁₂ IN METFORMIN TREATED TYPE 2
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ABSTRACT

Objective: To analyze serum homocysteine and vitamin B₁₂ in type 2 diabetics taking metformin therapy. **Study Design:** Cross sectional study design. **Study setting & Duration:** Department of Medicine, Liaquat University Hospital Jamshoro/Hyderabad from august 2017 to March 2018. **Subjects and Methods:** A sample of 100 volunteers; 50 controls (type 2 diabetics) and 50 cases (type 2 diabetics taking metformin), was selected according to selection criteria. Venous blood samples were collected under aseptic conditions. Random blood glucose, glycated Hb A (HbA1c), serum homocysteine and vitamin B₁₂ were detected. Data was analyzed on SPSS ver 22.0 (IBM, Incorporation, USA) at 95% Confidence interval ($P \leq 0.05$). **Results:** Elevated serum homocysteine was noted 48% in cases compared to 6% in controls ($P=0.0001$). Vitamin B₁₂ deficiency in controls was noted as 38% versus 64% in cases taking metformin therapy ($P=0.0001$). Serum homocysteine and Vitamin B₁₂ in controls and cases was noted as $6.71 \pm 2.82 \mu\text{M/L}$ and $15.35 \pm 11.97 \mu\text{M/L}$ & $239.54 \pm 61.72 \text{ pg/dl}$ and $197.40 \pm 76.23 \text{ pg/dl}$ respectively ($P=0.0001$). Vitamin B₁₂ shows negative correlation of vitamin B₁₂ with serum homocysteine ($r = -0.629$, $P = 0.0001$). **Conclusion:** The present study reports vitamin B₁₂ deficiency in 64% and elevated homocysteine in 48% type 2 diabetics taking metformin therapy.

KEYWORDS: Homocysteine, Vitamin B₁₂, Metformin, Type 2 Diabetics.**INTRODUCTION**

Prevalence of Diabetes mellitus (DM) is reported as 7.7% in rural areas and 10.6% in urban areas in Pakistan. Total diabetic population is reported as 7.2 million approximately.^[1] Metformin is an orally taken biguanides anti-diabetic drugs widely used throughout the World for the type 2 DM (T2DM). Approximately, 120 million diabetics are prescribed metformin globally.^[2] Metformin is in use since past 60 years. It is prescribed as a first line oral anti-diabetic drug. Extensively prescription is due to its proven therapeutic efficacy and cost effectiveness.^[3] However, its use is associated with adverse drug effects such as vitamins deficiencies in particular the Vitamin B₁₂ (cobalamin deficiency). Cobalamin deficiency is one of few but serious side effects of metformin therapy because this vitamin catalyzes vital biochemical reactions and is essential for the nerve integrity. Vitamin B₁₂ is one of highly overlooked side effects. This is denied owing to the high therapeutic efficacy of metformin.^[4] Health providers deny vitamin B₁₂ levels in metformin treated diabetics putting the patients at increased risk of neuropathy. Vitamin B₁₂ is an important cause of

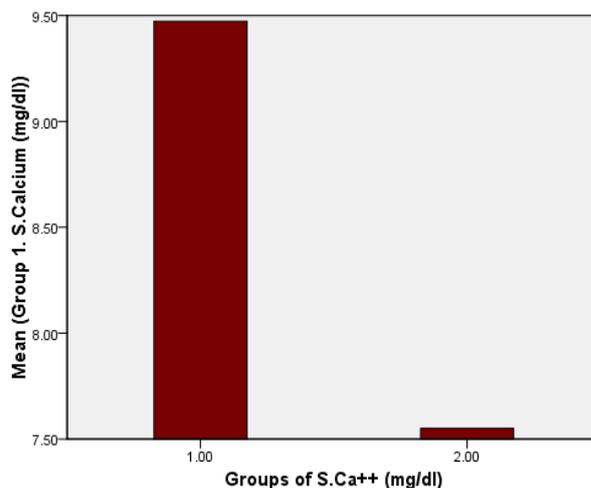
macrocytic megaloblastic anemia and bone marrow cell maturation arrest.^[5] This is because the vitamin B₁₂ is vital for the nuclear maturation of rapidly proliferating cells. Vitamin B₁₂ is involved in one carbon methylation reactions of DNA biosynthesis and cell metabolism. Hence DNA disruption, altered cell metabolism and cell maturation are hallmarks of its deficiency. This may lead to clinical consequences that become grave in severe deficiency states. Inside the cell, vitamin B₁₂ is converted into 2 active co-enzymes; the SAC (S adenosyl cobalamin) in mitochondria and MC (methylcobalamin) in the cytoplasm. SAC and MC play role in the homeostasis of homocysteine (HC) and methyl malonic acid (MMA) in the cells, tissues, and blood.^[6] HC and MMA are sensitive markers of vitamin B₁₂ deficiency. HC and MMA indicate vitamin B₁₂ deficiency better than the vitamin B₁₂ blood levels.^[7] Sensitivity of HC and MMA is reported as 99.8% for diagnosing vitamin B-12 deficiency.^[8] Diabetes mellitus is increasing in Pakistan and metformin is widely prescribed anti diabetic drug. However, the screening of vitamin B₁₂ levels is highly neglected issue that remains undiagnosed due to asymptomatic course.^[1,9] The present prospective study

was conducted to determine serum homocysteine and vitamin B₁₂ in type 2 diabetics taking metformin therapy reporting at a tertiary care hospital of Sindh.

SUBJECTS AND METHODS

The present cross sectional study was conducted at the Department of Medicine, Liaquat University Hospital Jamshoro/Hyderabad from august 2017 to March 2018. Written study approval was taken from the ethical review committee of the institute. A sample of 50 controls (type 2 diabetics) and 50 cases (type 2 diabetics taking metformin) was selected according to selection criteria. Controls comprised 38 male and 12 female while cases comprised of 36 male and 14 female subjects. Inclusion criteria were; diagnosed type 2 diabetics of >5 years duration, age 30- 50 years, both genders, metformin for \geq 6 months and metformin dose of 1- 2 grams a day. Type 2 diabetics suffering from concomitant major systemic disease such as chronic viral hepatitis, liver cirrhosis, cardiac failure, chronic inflammatory diseases, pulmonary tuberculosis and malabsorption syndromes were excluded. Strict vegetarians, pregnant female, and subjects taking multivitamin pill therapy, H₂ blockers and Proton pump inhibitors were also excluded. Medical officers were negotiated for help for proper screening of patients according to the inclusion and exclusion criteria. Complete biodata, duration of Diabetes, metformin therapy, and multivitamin pills. Diabetic volunteers were communicated by the concerned researcher and medical officers of medical wards and outpatient departments. 50 diabetic volunteer cases (taking metformin therapy) were selected according to inclusion criteria. And 50 diabetic controls – not taking metformin were selected. Controls were age and gender matched. A pre- structured pre-designed clinical proforma was designed for data collection. Proforma was available for the filling of biodata, clinical findings such as age and gender, random blood glucose, glycated Hb A (HbA1c), serum homocysteine and vitamin B₁₂. Participants were informed of the study purpose. They were informed that the study will cause no harm to them. Volunteers were asked to sign the consent form. Confidentiality of data was secured. Volunteers were asked for blood sampling from ante cubital fossa after proper aseptic measures. Prepared alcohol swabs were used for area to be cleaned for venepuncture. Disposable BD syringes were used for blood sampling. 5 ml of blood was collected in EDTA and gel tubes. Blood glucose was estimated by glucose oxidase method and HbA1c by colorimetric method on Hitachi Chemistry analyzer. Serum homocysteine and vitamin B₁₂ were detected by ELISA assay kits. Serum homocysteine <15 μ M/L was taken as normal. Elevated serum homocysteine was defined as >15 μ M/L. Normal, borderline, deficiency and severe deficiency of vitamin B₁₂ were taken at >240pg/ml, 170-240 pg/ml, <170 pg/ml and <100 pg/ml respectively.^[10] Statistical analysis was performed on SPSS 22.0 (for windows release). Normal Gaussian distribution of continuous variables

was detected by Shapiro-Wilk test. Student's t-test was used for the continuous variable (age, serum homocysteine, Vitamin B₁₂) analysis and Chi-square test analysed the categorical variables (gender and Vitamin B₁₂ categories). Correlation was analyzed of vitamin B₁₂ with serum homocysteine, random blood sugar and HbA1c. Data was analyzed at 95% Confidence interval ($P \leq 0.05$).



RESULTS

Mean \pm SD age of controls and cases was noted as 47.64 \pm 7.76 and 47.50 \pm 7.58 years respectively ($P=0.79$). Male and female were noted as 76% and 24% in controls and 72% and 28% in cases respectively ($P=0.91$). HbA1c and Random blood sugar (RBS) show bad glycemic status in both controls and cases as shown in table I. Serum homocysteine was noted as 6.71 \pm 2.82 μ M/L in controls and 15.35 \pm 11.97 μ M/L in cases ($P=0.0001$). Vitamin B₁₂ deficiency was noted in cases 197.40 \pm 76.23 pg/dl compared to 239.54 \pm 61.72 pg/dl in controls ($P=0.0001$) (Table I). Vitamin B₁₂ categories and serum homocysteine levels are shown in table II and III. Pearson's correlation shows negative correlation of vitamin B₁₂ with serum homocysteine ($r = -0.629$, $P = 0.0001$) as shown in table IV and scatter plot 1. Vitamin B₁₂ showed statistically non- significant correlation with RBS ($P=0.611$) and HbA1c ($P=0.0861$) (Scatter plots 1 and 2). Vitamin B₁₂ deficiency in controls was noted as 38% versus 64% in cases taking metformin therapy (table II). Elevated serum homocysteine was noted 48% in cases compared to 6% in controls (table III).

Table I: Comparative findings of study subjects (n=100).

	Controls	Cases	P-value
Male	38 (76%)	36 (72%)	0.91
Female	12 (24%)	14 (28%)	
Age (years)	47.64±7.76	47.50±7.58	0.79
HbA1c (%)	10.48±3.57	10.16±3.26	0.921
RBS (mg/dl)	204.18±74.29	244.58±61.32	0.076
Homocysteine (µM/L)	6.71±2.82	15.35±11.97	0.0001
Vitamin B ₁₂ (pg/dl)	239.54±61.72	197.40±76.23	0.0001

Table II: Frequency of Vitamin B₁₂ in study subjects (n=100).

Vitamin B ₁₂ levels	Controls	Cases	P-value
Normal (>240 pg/ml)	31 (62%)	18 (36%)	0.023
Borderline deficiency (170-240 pg/dl)	8 (16%)	8 (16%)	
Deficiency (<170 pg/dl)	11 (22%)	21 (42%)	
Severe deficiency (<100 pg/dl)	0 (%)	3 (%)	
Total	50	50	

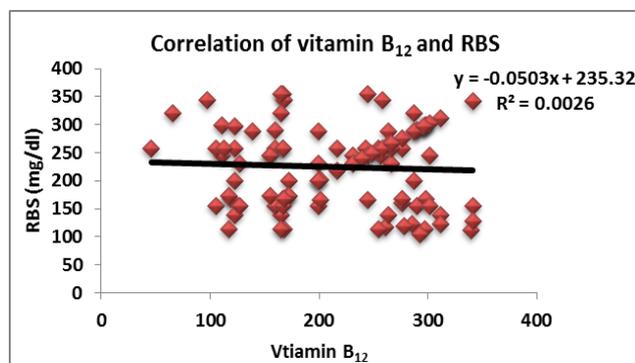
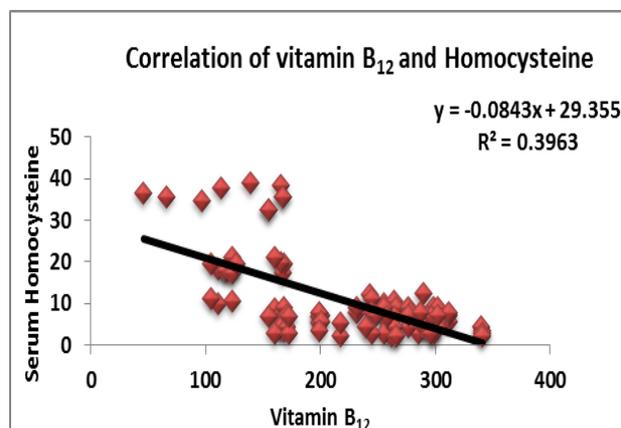
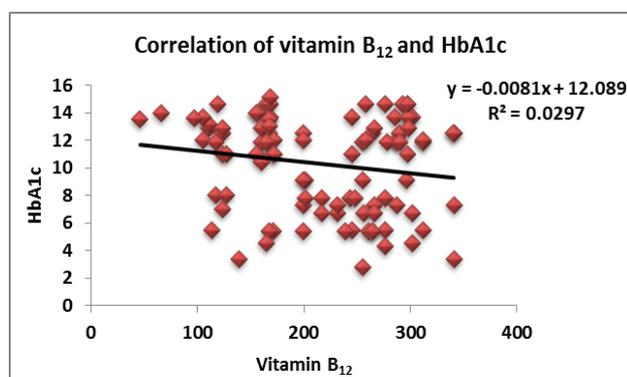
Table III: Frequency of serum homocysteine in study subjects (n=100).

Serum Hcy levels	Controls	Cases	P-value
Normal Hcy levels (<15µM/L)	47 (54%)	26 (52%)	0.0001
Elevated Hcy levels (>15µM/L)	3 (6%)	24 (48%)	
Total	50	50	

Table IV: Correlation of vitamin B12 in study subjects (n=100).

	RBS (mg/dl)	HbA1c (%)	Hcy* (µM/L)
Pearson Correlation	-0.05	-0.172	-0.629**
P- value	0.611	0.0861	0.0001

**Correlation is significant at the 0.01 level (2-tailed), *Hcy – homocysteine

Graph 1: Scatter plot showing correlation of vitamin B₁₂ and Random blood sugar.Graph 3: Scatter plot showing correlation of vitamin B₁₂ and Homocysteine.Graph 2: Scatter plot showing correlation of vitamin B₁₂ and HbA1c.

DISCUSSION

The present cross sectional is first time reporting on the frequency of serum homocysteine and vitamin B₁₂ deficiency in type 2 diabetics taking metformin therapy. The mean ± SD age of controls and cases was noted as 47.64±7.76 and 47.50±7.58 years respectively. Male and female were noted as 76% and 24% in controls and 72% and 28% in cases respectively. These findings are in agreement with previous studies.^[11-13] Vitamin B₁₂ deficiency in controls was noted as 38% versus 64% in cases taking metformin therapy. Elevated serum homocysteine was noted 48% in cases compared to 6%

in controls. These findings are consistent with previous studies,^[13,14] but inconsistent to a recent study.^[15] Adnan et al,^[15] has reported vitamin B₁₂ deficiency in 29.66% of type diabetic subjects taking metformin therapy. The reason of this discrepancy could be different study population. Pearson's correlation shows negative correlation of vitamin B₁₂ with serum homocysteine ($r = -0.629$, $P = 0.0001$) (table IV and scatter plot 1). Vitamin B₁₂ showed statistically non-significant correlation with RBS ($P = 0.611$) and HbA1c ($P = 0.0861$) (Scatter plots 1 and 2). These findings are concordant to a recent study.^[16] The findings of present study are comparable to previous studies, however the underlying mechanism of how metformin causes vitamin B₁₂ deficiency is not well established.^[17-19] Hence the topic is debatable and needs rigorous research on the exact mechanism of how vitamin B₁₂ deficiency is created by the metformin therapy. Jager et al,^[20] reported high frequency of vitamin B₁₂ deficiency in their randomized clinical trial conducted over 4.3 years duration. They reported 19% drop in vitamin B₁₂ levels per year ($P < 0.001$). Another previous study^[21] reported long term metformin therapy is associated with vitamin B₁₂ deficiency with elevated serum homocysteine. They suggested multivitamin supplements for type 2 diabetics taking metformin. The finding of vitamin B₁₂ deficiency and elevated serum homocysteine are in agreement with above study. The findings of elevated serum homocysteine and vitamin B₁₂ deficiency are supported by a previous study.^[22] Cassinadane et al,^[16] reported low vitamin B₁₂ in metformin treated type 2 diabetics and negative correlation with elevated serum homocysteine. These findings are in full agreement with present study. The present study reports negative correlation of vitamin B₁₂ with serum homocysteine ($r = -0.629$, $P = 0.0001$). In present study the serum homocysteine was noted as $6.71 \pm 2.82 \mu\text{M/L}$ in controls compared to $15.35 \pm 11.97 \mu\text{M/L}$ in cases ($P = 0.0001$). Vitamin B₁₂ in cases was noted as $197.40 \pm 76.23 \text{ pg/dl}$ compared to $239.54 \pm 61.72 \text{ pg/dl}$ in controls ($P = 0.0001$), the findings corroborate with a recent study by Raizada et al.^[23] They,^[23] reported serum Vitamin B₁₂ levels in metformin group as $267.7 \pm 194.4 \text{ pmol/l}$ versus no metformin group as $275.1 \pm 197.2 \text{ pmol/l}$. These findings are in keeping with present study. The evidence based findings of present study show elevated serum homocysteine and low vitamin B₁₂ in metformin treated type 2 diabetics. The limitations of present study are; first- small sample size, second- short duration of metformin therapy, third- peculiar ethnicity. However, the strength of study is because of prospective study design and inclusion and exclusion criteria, hence further research is advised to explore the true burden of problem of vitamin B₁₂ deficiency and elevated serum homocysteine in type 2 diabetics taking metformin therapy.

CONCLUSION

The present study reports vitamin B₁₂ deficiency in 64% and elevated homocysteine in 48% type 2 diabetics taking metformin therapy. Vitamin B₁₂ shows negative

correlation with serum homocysteine. Clinical practitioners must pay attention to serum vitamin B₁₂ and homocysteine levels in metformin treated type 2 diabetics.

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