



ORIGINAL ARTICLE

## Gender based deficiency of vitamin B12 in patients on metformin for diabetes mellitus.

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**ABSTRACT... Objective:** To determine deficiency of vitamin B12 among patients on metformin due to type II diabetes mellitus. **Study Design:** Cross Sectional Observational study. **Setting:** Department of General Medicine, Naz Memorial Hospital, Karachi. **Period:** April 2020 to March 2021. **Material & Methods:** After ethical approval and informed consent patients of type II diabetes on single oral hypoglycemic drug metformin for at least six months, in-between 25-60 years of age, on 850 mg metformin twice a day were included. Patients with history of metformin use in addition to another oral hypoglycemic drug were excluded. SPSS was used for data analysis. Quantitative data was expressed as mean and standard deviation and qualitative variables as frequency and percentages. Chi-square test was applied keeping p-value <0.05 as statistically significant. **Results:** Among 240 patients, mean age of patients was  $44.62 \pm 5.5$  years with a mean BMI of  $30.91 \pm 2.74$  kg/m<sup>2</sup>. Mean hemoglobin was  $11.5 \pm 1.3$  g/dl and mean hematocrit of  $34.77 \pm 5.58\%$ , mean glycosylated hemoglobin  $7.34 \pm 0.73\%$ , mean duration of diabetes mellitus  $2.60 \pm 0.7$  years and mean use of metformin was  $1.99 \pm 0.7$  years. Mean level of vitamin B12 was  $240 \pm 76.6$  pg/ml. Normal vitamin B12 level, >300 pg/ml was observed in 41 (17.1%), borderline level in 97 (40.4%) while deficiency in 102 (42.5%) of patients. **Conclusion:** Majority of patients were found to have either borderline or deficiency of vitamin B12. Furthermore, amongst the patients having vitamin B12 deficiency, a significant difference in-between the deficiency of vitamin B12 was found among females when compared to males.

**Key words:** Metformin, Type II Diabetes Mellitus, Vitamin B12.

### INTRODUCTION

Diabetes mellitus encircles metabolic disorders which are characterized by hyperglycemia caused by insulin secretion, action, or both. Diabetes causes long-term effects, organ dysfunction and organ such as in kidneys, eyes, blood vessels, heart and nerve.<sup>1</sup> Most frequently administered oral hypoglycemic drug include metformin, being the first line of drug which has been the recommendation of European Association for Study of Diabetes (EASD) as well as American Diabetic Association (ADA), and by other global clinical practitioners. If there are no other contraindications, metformin should be prescribed as the first choice of drug after diagnosis, along with lifestyle changes.<sup>2</sup> Most of the time, metformin causes mild symptoms such as GIT symptoms like abdominal discomfort,

diarrhea or soft stools. Such symptoms present soon after starting metformin whilst they tend to disappear when once stopping metformin.<sup>3</sup> Long-term use of metformin for treatment can result in vitamin B12 deficiency as reported in a few studies, which is not easily detected and requires close monitoring. Different researches have observed serum level of vitamin B12 is inversely proportional to dose and duration of using metformin.<sup>4</sup> According to some studies, 10-30% of diabetics have some sort of vitamin B12 malabsorption. Even though it has remained a matter of great debate as to whether deficiency of vitamin B12 has any significant association with metformin's use, nonetheless screening and monitoring levels of vitamin B12 is advised for type II diabetics especially those which are on metformin treatment for long.<sup>5</sup>

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A deficiency of vitamin B12 can result in altered mental health, megaloblastic anemia, and neurodegeneration. Diabetic neuropathy, peripheral neuropathy caused by CNS overlapping with paresthesia, impaired vibration sense, and proprioception can also be associated with diabetic neuropathy.<sup>6</sup> Ageing, genetic disorders of osteogenesis, a lack of physical activity, and estrogen deficiency in postmenopausal women are thought to be reasons behind vitamin B12 deficiency especially in patients on metformin, among other factors. Dietary risk factors are especially important because they can be altered.<sup>7,8</sup> The conversion of homocysteine (HCY) to methionine requires methylfolates as a methyl donor and vitamin B12 as a cofactor. Vitamin B12 deficiency, which can lead to an increase in homocysteine and cysteine concentrations, the two amino acids have also been associated with other systemic disorders. Vitamin B12 has also been linked to osteoblast activity and bone formation.<sup>9</sup> Furthermore, patients with pernicious anemia have been associated with increased risk of fracture. The proposed mechanisms behind deficiency of vitamin B12 among patients on metformin is that in diabetic patients, bowel motility is known to be altered in terms of bacterial overgrowth, leading to vitamin B12's deficient absorption. In addition, metformin causes inhibition of calcium dependent vitamin B12-intrinsic factor's absorption from the last part of ileum causing deficient absorption of vitamin B12 as well as intrinsic factor.<sup>10</sup> This inhibition can be returned to normal through supplementation of calcium.<sup>11</sup>

The aim of the study is to determine frequency of deficiency of vitamin B12 in patients on Metformin for Diabetes Mellitus.

## MATERIAL & METHODS

This cross sectional observational study, by employing non-probability convenient sampling technique was done at Department of General Medicine of Naz Memorial Hospital for one year (April 2020 to March 2021). After ethical approval from IRB (ERC/NMH/144/09) and informed consent from patients, data collection was done. Selection of patients was done in which inclusion

criteria consisted of diagnosed type II diabetics patients on a single oral hypoglycemic drug viz Metformin for at least six months, patients above the age of 25 years but below 60 years with a body mass index (BMI) of 22 kg/m<sup>2</sup> and above on 850 mg of metformin twice a day were included. Patients with a history of Metformin use in addition to another oral hypoglycemic drug were excluded along with patients having a history of abdominal or bariatric surgery which could affect vitamin B12 absorption and patients with a co-morbid disease such as cardiovascular, renal, metabolic syndrome, gastric disorders were excluded from the study.

Deficiency of vitamin B12 was termed as level <200 pg/ml while borderline levels were defined in-between 201-300 pg/ml. Hemoglobin and hematocrit were checked as safety measures for detecting vitamin B12 deficiency anemia among the patients. Anemia was defined as hemoglobin levels <12 g/dl or hematocrit <36% for females and <13 g/dl or <40% for males respectively.

For analysis of data, SPSS version 23.0 was used. Quantitative variables such as age, BMI, HbA1c, hemoglobin, hematocrit and vitamin B12 levels were represented as mean and standard deviation. Qualitative variables included gender and patients with anemia were expressed as frequency and percentages. To test for association between vitamin B12 levels and gender, chi-square test was applied keeping p-value of <0.05 as statistically significant.

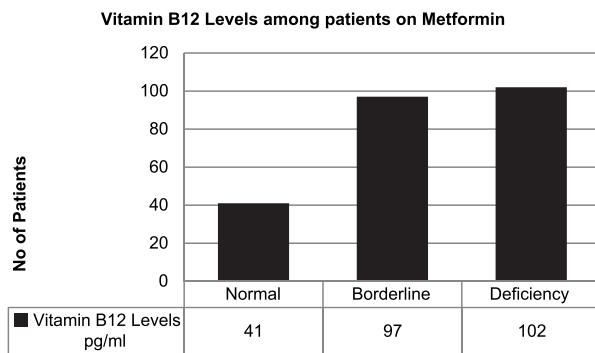
## RESULTS

According to results of study, from the total of 240 patients included, mean age of patients was  $44.62 \pm 5.5$  years with a mean BMI of  $30.91 \pm 2.74$  kg/m<sup>2</sup>. The mean hemoglobin concentration among the patients was  $11.5 \pm 1.3$  g/dl and mean hematocrit of  $34.77 \pm 5.58\%$ . The mean glycosylated hemoglobin level were  $7.34 \pm 0.73\%$ . Mean duration of diabetes mellitus of the patients was  $2.60 \pm 0.7$  years while the mean use of Metformin was  $1.99 \pm 0.7$  years. The mean level of vitamin B12 in the patients was  $240 \pm 76.6$  pg/ml [Table-I].

Variables	Mean ± Standard Deviation (n=240)
Age (years)	44.62 ± 5.5
BMI (kg/m <sup>2</sup> )	30.91 ± 2.74
HbA1c (%)	7.34 ± 0.73
Hemoglobin (g/dl)	11.5 ± 1.3
Hematocrit (%)	34.77 ± 5.58
Diabetes years	2.60 ± 0.7
Metformin use (years)	1.99 ± 0.7
Vitamin B12 (pg/ml)	240 ± 76.6

**Table-I. Baseline demographics of patients on metformin for diabetes mellitus.**

Amongst the patients, a normal level of vitamin B12, viz >300 pg/ml was observed in 41 (17.1%) of patients. A borderline level of vitamin B12, i.e. in-between 201-299 was seen in 97 (40.4%) while deficiency of vitamin B12 was reported in 102 (42.5%) of patients [Figure-1].



**Figure-1. Graphical representation of frequency of patients with vitamin B12 deficiency.**

In terms of association of vitamin B12 according to gender, a normal level of vitamin B12 was seen in 24 (58.5%) out of 41 patients in normal vitamin B12 level group while 17 (41.5%) of females were found to have normal vitamin B12 level. A significant association of  $p < 0.001$  was observed in-between the group. Among 97 patients with borderline vitamin B12 levels, 58 (59.8%) of patients were male while 39 (40.2%) of patients were female having a significant association of  $p < 0.001$  in-between them. Amongst the 102 patients with deficient vitamin B12, 28 (27.5%) were males while 74 (72.5%) were females. A highly substantial association of  $p < 0.001$  was observed in-between the group  $p < 0.001$  [Table-II].

Variables	Male (n=110)	Female (n=130)	P-Value
Normal Vitamin B12 (n=41)	24 (58.5%)	17 (41.5%)	<0.001
Borderline Vitamin B12 (n=97)	58 (59.8%)	39 (40.2%)	<0.001
Deficient Vitamin B12 (n=102)	28 (27.5%)	74 (72.5%)	<0.001

**Table-II. Association of vitamin B12 levels according to gender. Chi-square test applied to test for association**

**DISCUSSION**

Studies have demonstrated that among diabetic patients taking metformin for long term show some sort of deficiency from vitamin B12.<sup>12</sup> It has been shown that age, gender, BMI, status of diabetes (uncontrolled), hemoglobin and hematocrit all tend to have association with vitamin B12 levels among metformin users.<sup>13</sup> Similarly, the results of our study showed that a high incidence of vitamin B12 deficiency was observed in patients on metformin for diabetes. From total patients, 102 (42.5%) of patients in our study were found to have significant deficiency of Vitamin B12. Furthermore, such patients were also observed to have an uncontrollable level of glycemic index i.e. HbA1c along with low hemoglobin as well as hematocrit.

The reported frequency of vitamin B12 deficiency among adults and elderly diabetics has been estimated at about 20%, even though the incidences greatly vary, depending upon study population as well as the cut off value of B12 used.<sup>13</sup> A variety of factors have been attributed to it such as dietary habits, physiology of gastrointestinal tract, associated autoimmune disease and use of medicines.<sup>14</sup> In one of the study carried out to determine the deficiency of Vitamin B12 levels among patients on biguanide therapy and metformin therapy among diabetics, reported higher proportion of patients, 71 patients on metformin as compared to 46 patients using biguanides.<sup>15</sup>

In yet another study done for assessing risk of deficiency of vitamin B12 with use of metformin

among Diabetes Prevention Program (DPP) / DPP Outcomes Study (DPPOS) observed that low levels of vitamin B12 (<200 pg/ml) were reported more commonly in the metformin group when used for long term. Besides vitamin B12 deficiency, anemia and neuropathy were also more frequently found in metformin group in comparison to placebo group. The research concluded long-term metformin use can be linked to anemia and vitamin B12 deficiency. It also recommended that routine vitamin B12 testing among metformin treated patients must be considered. However the study did not shed light on the pathology behind it.<sup>16</sup>

Several studies have unequivocally termed metformin the prime factor behind vitamin B12 deficiency amongst type II diabetics.<sup>17</sup> The range of vitamin B12 deficiency's prevalence has been predicted in-between 6 to 45%.<sup>18</sup> In our research, reported frequency of vitamin B12 deficiency was 42.5%. The probable variation in such a wide prevalence can be attributed to the study population a study definitions used for vitamin B12. The relationship between metformin induced vitamin B12 deficiency has been partly attributed towards increase in age, dosage and duration of metformin use. Increased dose and longer duration of metformin use has been linked towards vitamin B12 deficiency.<sup>19</sup> A Chinese research on metformin usage among diabetic patients reported that in every 1 gm/ day rise of metformin dose demonstrated an increase in 2.9 time chances for developing of vitamin B12 deficiency.<sup>20</sup> Another study observed that with the start of metformin, as early as from four months, decreased absorption of vitamin B12 starts to take place. However clinically proven vitamin B12 deficiency occurs after at least 2-5 years, even after 10 years sometimes. Likewise in our study as well, the mean duration of metformin use was greater than 2 years, falling into the above category.<sup>21</sup>

The mechanisms proposed in explaining vitamin B12 deficiency amongst metformin users having type II diabetes are; an altered motility of the small bowel that tends to stimulate overgrowth of bacteria and consequently leading to deficiency

of vitamin B12, either through competitive inhibition or by inactivating absorption of vitamin B12, altered levels of intrinsic factor (IF) and interaction with endocytic receptors.<sup>22</sup> It has been found that metformin may inhibit calcium dependent absorption of vitamin B12-IF complex in terminal part of ileum. Such inhibitory effect can be reversed through supplementation of oral or parenteral calcium.<sup>23</sup>

In contrast to our study in which higher frequency of females were observed to have vitamin B12 deficiency, another research found that prevalence of vitamin B12 deficiency was higher among males (25.5%) in comparison to the prevalence observed among females (18.9%). Nonetheless, females were strongly linked with severity of vitamin B12 deficiency when compared to males. The study concluded that the disparity of vitamin B12 deficiency among gender cannot be explained by either dietary habits or by the effects of male and female sex hormones such as testosterone and estrogen on vitamin B12 levels as well as with the use of metformin. However, genetic variation can be hypothesized to impart a role in such cases.<sup>24</sup>

The results of our study showed that the prevalence of vitamin B12 among metformin users for diabetes was high, being reported in higher frequencies among females. In addition to vitamin B12 deficiency, decreased hemoglobin and hematocrit were also found. Nevertheless, the study was not immune from selection and observer bias and the fact that the study was conducted at a single center with limited sample size. Furthermore we also did not compare use of metformin a different dosages nor compared to it with other medications or placebo and even with a controlled group. Further, multi-centered studies with greater sample size and comparing the long term effect of metformin at different dosages or with other drugs/ placebo/ controlled group would aid in authenticating the results reported in the current study.

## CONCLUSION

According to the study results, majority of type II diabetic patients on metformin were found to have

either borderline or a deficient level of vitamin B12. Furthermore, amongst the patients having vitamin B12 deficiency, a significant difference in-between the deficiency of vitamin B12 was found among females when compared to males.






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2	Ali Sarfaraz	Concept & Design of study.	
3	Kanwal Ijaz	Analysis & Interpretation of data.	
4	Tahir Mahmud	Literature review.	
5	Huda Abbas	Literature review.	
6	Nadia Tufail	Critical review of article.	