

Association of Insulin Resistance with Vitamin B₁₂ Status in Type 2 Diabetes Mellitus Patients on Metformin Therapy

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ABSTRACT

Introduction: Association of insulin resistance with obesity and metabolic syndrome is an established fact. Vitamin B₁₂ deficiency resulting from metformin therapy in type 2 diabetes mellitus (T2DM) patients was documented in the literature. In our study, we tried to explore the association of insulin resistance with vitamin B₁₂ deficiency in T2DM patients on metformin therapy.

Materials and methods: A total of 120 T2DM patients on metformin for at least 6 months with minimum dose of 1,000 mg/day between age group 35 years and 55 years were included. They were divided into group I (with B₁₂ deficiency) and group II (with normal B₁₂ levels). Patients who were on multivitamin supplementation or B₁₂ therapy, nutritional deficiency of vitamin B₁₂, or with any coexisting cause of B₁₂ deficiency like alcoholism, liver, thyroid, and cardiac diseases were excluded from the study. Following biochemical parameters were estimated: fasting plasma glucose, fasting plasma insulin, and vitamin B₁₂. Homocysteine and HOMO-IR were calculated. The statistical analysis, such as Student's *t*-test and Pearson correlation test, was performed.

Results: B₁₂ deficiency (group I) showed significant increase in insulin resistance. A negative correlation was observed between vitamin B₁₂ and HOMA IR. Homocysteine showed positive correlation with HOMA IR.

Conclusion: There is a negative correlation between the levels of vitamin B₁₂ and insulin resistance and a positive correlation between homocysteine and insulin resistance in T2DM patients on metformin therapy.

Keywords: Diabetes mellitus, Insulin resistant, Metformin, Vitamin B₁₂.

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INTRODUCTION

Over the past two decades, there is a rapid increase in the prevalence of diabetes among the Asian Indians. In 1995, there were around 19 million diabetics in India, which has considerably increased to 32 million in 2000 and 66.8 million in 2014.¹⁻³ Further, it is predicted to touch 100 million by 2030.

The significance of both β -cell dysfunction and insulin resistance in the production of hyperglycemia of type 2 diabetes mellitus (T2DM) is apparent.⁴ Insulin resistance, a condition occurring in muscles, fat, and liver cells, is also increasing rapidly among Indians.

Association of insulin resistance with obesity and metabolic syndrome is an established fact.^{5,6} Vitamin B₁₂ deficiency resulting from metformin therapy in T2DM patients was documented in the literature.^{7,8} In our study, we tried to explore the association of insulin resistance with vitamin B₁₂ deficiency in T2DM patients on metformin therapy.

MATERIALS AND METHODS

The study population included 120 T2DM patients on metformin for at least 6 months with minimum dose of 1000 mg/day between the age group 35 years and 55 years attending a tertiary care hospital in Puducherry. Institutional ethical committee clearance was obtained before patients were selected according to the inclusion and exclusion criteria. They were divided into group I (with B₁₂ deficiency) and group II (with normal B₁₂ levels). Patients who were on multivitamin supplementation, B₁₂ therapy, nutritional deficiency of vitamin B₁₂, or with any coexisting cause for B₁₂ deficiency like alcoholism, liver, thyroid, and cardiovascular diseases were excluded from the study.

Fasting plasma glucose was estimated by the glucose oxidase peroxidase method (reference range 70–110 mg/dL), fasting plasma

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insulin (ref. range: 5–17 μ unit/mL) and vitamin B₁₂ (ref. range: 200–835 pg/mL) by the chemiluminescence method, homocysteine by the immunoturbidimetry method (ref. range: 4–14 μ mol/L), and homeostatic model assessment of insulin resistance (HOMO-IR, ref. range: 0.5–1.4) was calculated by using the formula fasting insulin \times fasting glucose/405.

The Kruskal–Wallis test was done to analyze the normal distribution of data. After that the Student's *t* test was performed

to compare between groups. The Pearson correlation test was used for the correlation analysis. Significance was assessed at $p < 0.05$.

RESULTS

A total of 120 T2DM patients on metformin therapy was analyzed for their vitamin B₁₂ status and divided into two groups. Group I comprised of 49 patients with vitamin B₁₂ deficiency and group II had 71 patients with normal vitamin B₁₂ levels. Homocysteine was also analyzed as an added parameter to confirm the B₁₂ status. In group I, 24 (49%) were male and 25 (51%) were female. In group II, 28 (40%) were male and 43 (60%) were female as shown in Table 1.

Based on the vitamin B₁₂ status, the study population was divided into two groups. In Table 2, the insulin resistance marker HOMA-IR was compared between the groups. Subjects with vitamin B₁₂ deficiency (group I) showed significant increase in insulin resistance ($p < 0.01$). In addition, as a support for our study we compared homocysteine, a marker for vitamin B₁₂ deficiency levels between the groups, and it showed a significant increase in group I subjects.

A negative correlation was observed between vitamin B₁₂ and HOMA-IR ($r = -0.290$, $p = 0.037$). Homocysteine showed positive correlation with HOMA-IR ($r = 0.397$, $p < 0.01$).

DISCUSSION

Metformin, which is still remaining as the optimal drug for monotherapy with respect to T2DM, is said to induce vitamin B₁₂ deficiency.^{2,9} B₁₂ deficiency prevalence ranges from 6 to 30% among T2DM patients undergoing long-term treatment with metformin.^{10,11}

In our study of 120 T2DM patients who were on metformin therapy, 49 patients were found to be having vitamin B₁₂ deficiency, which amounts to around 41% of the study population. Previous studies showed a mean value of less than 221 pg/mL as vitamin B₁₂ deficient. Comparatively, in our study we had a mean value of 205 pg/mL in group I. In this case, the control study we identified that increased HOMA-IR is associated with low levels of vitamin

Table 1: Demographic data of the study groups

Demographics	Group I (n = 49)	Group II (n = 71)
Age	50.38 ± 4.52	46.76 ± 5.87
Male	24 (49%)	28 (40%)
Female	25 (51%)	43 (60%)

Group I = vitamin B₁₂ deficient; group II = normal B₁₂ levels

Table 2: Comparison of biochemical variables between group I and group II subjects

Parameters	Group I (mean ± SD)	Group II (mean ± SD)	p value
Fasting plasma glucose (mg/dL)	180.22 ± 31.57	180.43 ± 40.96	0.97
Fasting plasma insulin (mIU/L)	6.13 ± 2.02	8.41 ± 2.34	0.00*
HOMA-IR	3.72 ± 1.22	2.73 ± 1.04	0.00*
Vitamin B ₁₂ (pg/mL)	191.01 ± 43.67	540.16 ± 161.26	0.00*
Homocysteine (μmol/L)	25.23 ± 13.83	13.83 ± 4.5	0.00*

* $p < 0.001$ is statistically significant

B₁₂ and increased homocysteine in T2DM patients on metformin therapy.

The inverse relationship between vitamin B₁₂ and insulin resistance is in accord with a previous study.¹² Vitamin B₁₂ serves as a cofactor in methionine synthesis as well as in conversion of methyl malonic acid to succinylcholine.^{12,13} Hence, vitamin B₁₂ deficiency might affect insulin resistance by increasing the stress in the endoplasmic reticulum by the leakage of cellular folate, thereby leading to deficiency of free fatty acid oxidation. Simultaneously, the increase in methyl malonic acid (MMA) causes lipogenesis and insulin resistance as explained by Li et al.¹⁴

Homocysteine, a marker for vitamin B₁₂ deficiency, was elevated in group I, which showed a positive correlation with insulin resistance. The relationship between homocysteine and insulin resistance was explored in previous studies.¹⁵⁻¹⁷ In our study, we observed that metformin therapy leads to vitamin B₁₂ deficiency in 41% of the T2DM patients and they also had increased levels of homocysteine, which may be a confounding factor in increasing the insulin resistance.

We have found the associations of vitamin B₁₂ and homocysteine with insulin resistance among T2DM patients on metformin therapy. Further investigations might be essential to find out the underlying pathogenesis of insulin resistance in B₁₂ deficiency.

CONCLUSION

There is a negative correlation between the levels of vitamin B₁₂ and insulin resistance and a positive correlation between homocysteine and insulin resistance in T2DM patients on metformin therapy. Further studies are required to correlate insulin resistance with the dose and duration of metformin therapy together with the body mass index.

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