ORIGINAL ARTICLE

CORRELATION AND PREVALENCE OF MEGALOBLASTIC ANAEMIA IN TYPE 2 DIABETIC PATIENTS USING METFORMIN

Ghulam Farooq, Sidra Humayun*, Muhammad Ishfaq, Javaid Hassan***, Raveed Khan**, Hina Gul****

Department of Medicine, Peshawar Institute of Medical Sciences/Pak International Medical College, Peshawar, *Department of Pathology, Muhammad College of Medicine, Peshawar, **Department of Medicine, Hayatabad Medical Complex, Peshawar, ***Department of Physiology, Muhammad College of Medicine, Peshawar, Pakistan

Background: Metformin is the most widely used drug in the management of type 2 diabetic patients but its use is associated with vitamin B_{12} deficiency induced megaloblastic anaemia. However few studies in Pakistan have assessed this risk. This study was conducted to determine correlation and prevalence of megaloblastic anaemia in type 2 diabetics treated with metformin. **Methods:** This cross-sectional study was carried out in the Department of Medicine, Hayatabad Medical Complex, Peshawar from Dec 2021 to Nov 2022. A total of 156 type 2 diabetic patients who were taking metformin at least for the last 2 years, were included in the study. All these patients were evaluated for anaemia, raised Mean Corpuscular Volume (MCV) and vitamin B_{12} levels. **Results:** Out of 156 patients who participated in the study, 94 (60.25%) were male and 62 (39.75%) were female with a mean age of 57.56±8.5 years. Mean duration of diabetes was 7.37±3.61 years, whereas mean duration of metformin use was 5.85±2.9 years. Vitamin B_{12} deficiency was identified in 51 (32.69%) patients. These B_{12} deficient patients demonstrated a mean Hb of 9.8±1.4 g/dL. Hyper-segmented neutrophils and raised MCV (mean MCV 98.7±6.90 fl was also identified in them. There was a no correlation between serum B_{12} levels and dose and duration of metformin. **Conclusion:** Long term metformin use is significantly associated with B_{12} deficiency associated megaloblastic anaemia.

Keywords: Diabetes Mellitus, Metformin, $HbA1_C$, Vitamin B_{12}, Megaloblastic Anaemia

Pak J Physiol 2023;19(3):23–6

INTRODUCTION

The predominant variant of diabetes worldwide is type 2 diabetes mellitus, impacting 85–90% of the total diabetic population. While type 2 diabetes mellitus primarily affects older adults and young individuals, it can also manifest in children. This condition arises from the interplay of genetic and environmental factors. A significant genetic predisposition exists, and the risk escalates substantially when coupled with lifestyle factors such as hypertension, excessive weight or obesity, insufficient physical activity, and poor dietary habits. Initially, type 2 diabetes often responds to interventions like a healthful diet and regular physical exercise.[1,2] Nevertheless, with time, most of the type 2 diabetes patients require adjunctive oral medications, and a considerable portion necessitates insulin therapy.

Metformin, a member of the biguanide class of oral anti-diabetic medications, stands as the primary first-line choice for managing type 2 diabetes.[3] Among the array of anti-hyperglycaemic agents, metformin demonstrates a positive correlation with enhanced cardiovascular outcomes, mitigating the elevated cardiovascular risk that contributes to mortality in patients with type 2 diabetes mellitus.[4]

Metformin usage presents several drawbacks. It triggers an impairment in vitamin B_{12} absorption, consequently elevating the susceptibility to vitamin B_{12} deficiency.[5] Metformin therapy has the potential to induce a reduction in folic acid levels.[6] The concurrent reduction in both vitamin B_{12} and folate concentrations results in an escalation of homocysteine levels, an autonomous risk factor for cardiovascular ailments.[7] Compromised cognitive function and the onset of Alzheimer's disease have been associated with diminished serum vitamin B_{12} levels among elderly patients.[8] Connections have been established between vitamin B_{12} deficiency and microangiopathic haemolysis as well as narcolepsy.[9]

Numerous studies have substantiated the correlation between metformin consumption and vitamin B_{12} deficiency in individuals with type 2 diabetes. Nevertheless, only a limited subset of these investigations has evaluated the prevalence of vitamin B_{12} deficiency in type 2 diabetes patients undergoing metformin treatment.

The prevalence of megaloblastic anaemia caused by B_{12} deficiency among patients receiving long-term treatment with metformin was 41% in a study conducted by Owhin SO *et al*[10]. This anaemia related with prolonged metformin use is almost always overlooked and seldom investigated by physicians, usually attributing it to the disease itself.[11]

On account of the revealed correlation between use of metformin and megaloblastic anaemia due to vitamin B_{12} deficiency and the lack of data in north-western region of Pakistan, the objective of this

study was to establish the prevalence of megaloblastic anaemia among Type 2 Diabetes Mellitus (T2DM) patients taking long-term metformin, who were followed-up at a medical out-patient clinic in a tertiary healthcare setup in north-western Pakistan.

MATERIAL AND METHODS

This cross-sectional study was done over a period of one year, from Dec 2021 to Nov 2022, at the Department of Medicine, Hayatabad Medical Complex (HMC), Peshawar. Ethical approval from the institutional ethical committee was granted before initiating this study. The study included a sample of 156 individuals aged 40–70 years who had been diagnosed with type 2 diabetes and taking 2,000 mg metformin for a minimum of 4 years.[12] The participants were selected using non-probability consecutive sampling and were asked to provide informed, written consent before being included in study.

Any patient with a previous history of anaemia, malabsorption, history of alcohol use, renal failure, patients currently on parenteral or enteral nutritional supplements, prior transfusion (during the last 3 months), previous gastric or intestinal surgery, and thyroid illness were excluded from the study. After a thorough clinical examination and detailed review of existing medical record, patients with strict vegetarian diet, neurological, or psychiatric diseases, unstable cardiopulmonary, and any neoplastic disorder were also excluded from the study. Additionally, subjects taking amino-salicylic acid, proton pump inhibitors, calcium supplements, H_2 receptor antagonists, vitamin B_{12}, or colchicine during the previous three months were disqualified from study.

The medical records of every patient were assessed and a specially designed proforma which was made after thorough literature review, was utilized to record the demographic information like name, age, gender, duration of DM and metformin use. Blood samples were collected by trained phlebotomists applying standard WHO phlebotomy protocols and transported to the main laboratory of HMC for investigations.

A complete blood count (CBC) was performed on blood samples, employing Sysmex pocH-100i® Automated Hematology Analyzer to look for anaemia (here defined as Hb<11 g/dL) and raised MCV (>96 fl). Parallel to this, the levels of serum vitamin B_{12} were determined using the Abbot Architect 1000 SR 239930 and the chemiluminescence technique. Serum B_{12} levels and haematological parameters were noted on the same proforma. Vitamin B_{12} levels below 200 pmol/L in patients were considered deficient, whereas levels above 300 pmol/L were regarded as normal.

SPSS-23 was used to analyse the gathered data and various tables, results, and calculations were made. To summarize the information, descriptive statistics were used, the variables of age, serum vitamin B_{12} levels, and duration of metformin use were considered as quantitative measures, and their Mean±SD were calculated. To evaluate the correlation between duration and dosage of metformin and serum vitamin B_{12} levels, Chi-square test was used with $p \leq 0.05$ considered as statistically significant.

RESULTS

All the participants were analysed based on age and gender distribution. The age ranged from 40–70 years with a mean age of 57.56±8.5 years. Male preponderance was noted with 94 (60.25%) male and 62 (39.75%) female. Mean duration of DM was 7.37±3.61 years whereas, mean duration of metformin use was 5.85±2.9 years.

The subjects demonstrated a mean $HbA1_C$ (glycosylated haemoglobin) of 8.85±1.5% while mean MCV was 93±4.67 fl/dL. Serum B_{12} levels ranged from 52 pmol/L to 920 pmol/L with mean B_{12} levels of 306±188.9 pmol/L, whereas vitamin B_{12} deficiency was identified in 51 (32.69%) patients.

Mean haemoglobin concentration of the patients under study was 12.65±2.4 g/dL whereas, B_{12} deficient patients (n=51) demonstrated a mean Hb of 9.8±1.4 g/dL.

Hyper-segmented neutrophils and raised MCV (mean MCV=98.7±6.90 fl) were also identified in them. The effect of metformin dose on cobalamin levels was assessed in patients taking less than 1.5 g and more than 1.5 g daily. Patients with low B_{12} levels received a mean dose of 2,100±510 mg, whereas patients with normal B_{12} levels received a mean dose of 1,570±628 mg. Serum B_{12} levels had inverse association with duration and dose of metformin.

Table-1: Demographic and laboratory features of study participants

Parameters	Frequency
Number of patients	156
Female	62 (39.75%)
Male	94 (60.25%)
Age (Years, Mean±SD)	57.56±8.50
Duration of DM (Years, Mean±SD)	7.37±3.61
$HbA1_C$ (%, Mean±SD)	8.85±1.5%
MCV (fl, Mean±SD)	93±4.6
Hb (g/dL, Mean±SD)	12.65±2.4
Vitamin B_{12} levels (pg/mL, Mean±SD)	306±190.5
Vitamin B_{12} deficiency	51 (32.69%)

Table-2: Clinical and laboratory characteristics of the individuals in relation to vitamin B_{12} levels

Demographics/ Parameters	Serum Vitamin B_{12} Levels		*p*
	Deficient <220 pg/ml	Normal >220 pg/ml	
Number of patients	51 (32.69%)	105 (67.31%)	
Age, (Years, Mean±SD)	56.01±7.65	55.98±8.11	0.04
Male	34 (66.66%)	60 (57.14%)	0.23
Female	17 (33.34%)	45 (42.86%)	0.42
Vitamin B_{12} levels, pg/ml (Mean±SD)	125±43.01	389±181.50	0.00001
MCV, fl (Mean±SD)	98.7±6.90	91±5.99	0.04
Hb, g/dL (Mean±SD)	9.8±1.4	12.5±1.8	0.04
Duration of DM, (Years, Mean±SD)	8.88±5.67	5.69±4.83	0.075
Duration of metformin use (Years, Mean±SD)	3.6±1.5	1.60±0.8	0.001
Dose of metformin, (mg, Mean±SD)	2042±540.73	1605±632.80	0.0003
$HbA1_C$, (%, Mean±SD)	8.83±0.90	8.33±0.88	0.405

DISCUSSION

Type 2 diabetes is a prevailing endocrine disorder on a global scale. Metformin is a widely prescribed drug for diabetes. Megaloblastic anaemia due to cobalamin (vitamin B_{12}) deficiency is one of the documented adverse effects of long-term metformin usage.[13] The transportation of vitamin B_{12}-intrinsic factor complex across the membrane of ileal cells is reliant on calcium, and the action of metformin on this calcium-dependent process can result in B_{12} deficiency and megaloblastic anemia.[14]

We found a significant number of diabetic patients with megaloblastic anaemia due to vitamin B_{12} deficiency (serum folate levels within normal limits). These patients were using metformin at least for the last two years and the blood smear showed hypersegmented neutrophils and raised MCV. Statistically significant correlation was observed between anaemia and B_{12} deficiency (p=0.045). These findings are supported by those of a study conducted on 34 Indian patients in 2018, identifying megaloblastic anaemia in 41% diabetics using metformin; similar results were obtained by other studies.[12,15,16]

We also correlated the effects of metformin dose and duration of its use with B_{12} deficiency. A significant inverse correlation was observed between B_{12} levels and dose and duration of metformin use (p=0.0003 and 0.001 respectively). These observations are comparable to another study by Marar *et al*, which showed a high prevalence of vitamin B_{12} deficiency in patients with T2DM on metformin therapy.[17] An inverse relationship was found between vitamin B_{12} levels and the dose and duration of metformin use as seen in study by Kim *et al*[18].

Demographic characteristics of our study subjects are comparable to that identified by other studies. Megaloblastic anaemia was more common in elderly diabetics probably due to imbalanced diet in elderly as compared to younger patients. Similar positive correlations between advancing age and B_{12} deficiency were found in another study by Kang *et al*[16].

Gender, $HbA1_C$, and B_{12} deficiency-related megaloblastic anaemia did not significantly correlate with one another.

CONCLUSION

There was a significant correlation between prolonged metformin usage and the development of secondary megaloblastic anaemia resulting from vitamin B_{12} deficiency. It is crucial for healthcare providers to acknowledge this important information and perform annual screenings for type 2 diabetic patients who have been using metformin for over two years. These screenings should involve laboratory tests such as complete blood count (CBC), peripheral smear examination, and serum vitamin B_{12} level assessment.

REFERENCES

1. Tinajero MG, Malik VS. An update on the epidemiology of type 2 diabetes: a global perspective. Endocrinol Metab Clin 2021;50(3):337–55.
2. Reed J, Bain S, Kanamarlapudi V. A review of current trends with Type 2 Diabetes epidemiology, aetiology, pathogenesis, treatments and future perspectives. Diabetes Metab Syndr Obes 2021;14:3567–602.
3. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137(1):25–33.
4. Zhang K, Yang W, Dai H, Deng Z. Cardiovascular risk following metformin treatment in patients with type 2 diabetes mellitus: Results from meta-analysis. Diabetes Res Clin Pract 2020;160:10800.
5. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, *et al*. Long-term metformin use and vitamin B_{12} deficiency in the diabetes prevention program outcomes study. J Clin Endocrinol Metab 2016;101(4):1754–61.
6. Owen MD, Baker BC, Scott EM, Forbes K. Interaction between metformin, folate and vitamin B_{12} and the potential impact on fetal growth and long-term metabolic health in diabetic pregnancies. Int J Mol Sci 2021;22(11):5759.
7. Ma Y, Peng D, Liu C, Huang C, Luo J. Serum high concentrations of homocysteine and low levels of folic acid and vitamin B_{12} are significantly correlated with the categories of coronary artery diseases. BMC Cardiovasc Disord 2017;17:37.
8. Cho HS, Huang LK, Lee YT, Chan L, Hong CT. Suboptimal baseline serum vitamin B_{12} is associated with cognitive decline in people with Alzheimer's disease undergoing cholinesterase inhibitor treatment. Front Neurol 2018;9:325.
9. Osman H, Alwasaidi TA, Al-Hebshi A, Almutairi N, Eltabbakh H. Vitamin B_{12} deficiency presenting with microangiopathic hemolytic anemia. Cureus 2021;13(1):e12600.
10. Owhin SO, Adaja TM, Fasipe OJ, Akhideno PE, Kalejaiye OO, Kehinde MO. Prevalence of vitamin B_{12} deficiency among metformin-treated type 2 diabetic patients in a tertiary institution, South-South Nigeria. SAGE Open Med 2019;7:2050312119853433.
11. Iftikhar R, Qadir A, Iqbal Z, Usman H. Prevalence of vitamin B_{12} deficiency in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. Pan Afr Med J 2014;16:67.
12. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, *et al*. Association of vitamin B_{12} and metformin in type 2 diabetes. J Korean Med Sci 2014;29(7):965–72.

13. Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B_{12} deficiency: An association to bear in mind. World J Diabetes 2021;12:916–31.
14. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B_{12} malabsorption induced by metformin. Diabetes Care 2000;23(9):1227–31.
15. Koduri VL, Nori SNS, Aditya SRKK, Madaboina S, Sindhuja ABK, Swati S. Haematological parameters and vitamin B_{12} levels in type II diabetic patients on metformin —a prospective case control study. J Evid Based Med Healthc 2018;5(31):2317–23.
16. Kang D, Yun JS, Ko SH, Lim TS, Ahn YB, Park YM, *et al.* Higher prevalence of metformin-induced vitamin B_{12} deficiency in sulfonylurea combination compared with insulin combination in patients with type 2 diabetes: a cross-sectional study. PLoS One 2014;9(10):e109878.
17. Marar O, Senturk S, Agha A, Thompson C, Smith D. The prevalence of vitamin B_{12} deficiency in patients with type 2 diabetes mellitus on metformin. R Coll Surg Irel Stud Med J 2011;4(1):16–20.
18. Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B_{12} deficiency in patients with type 2 diabetes. Medicine (Baltimore) 2019;98(46):e17918.

Address for Correspondence:
Dr Muhammad Ishfaq, Medicine C Unit, Hayatabad Medical Complex, Peshawar, Pakistan. **Cell:** +92-333-9005472
Email: dashfaq@gmail.com

Received: 25 Jul 2023 **Reviewed:** 27 Aug 2023 **Accepted:** 28 Aug 2023

Contribution of Authors:
BF: Principal Author, manuscript writing
SH: Sample collection and manuscript writing
MI: Sample Collection and Laboratory work
JH: Laboratory work and manuscript writing
RK: Result compilation and statistical analysis
HG: Data analysis and manuscript compilation

Conflict of Interest: None
Funding: None