The Effect of Metformin on Vitamin B12 Deficiency and Stroke

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ABSTRACT **Background:** Type 2 diabetes mellitus (T2DM) is a known risk factor for cardiovascular disease and stroke. Metformin is an old, relatively safe, first line therapy for T2DM; however, it has been associated with stroke.

Objectives: To study the effects of metformin use and vitamin B12 deficiency on stroke rate among patients with T2DM.

Methods: We conducted a prospective study of patients admitted with ischemic stroke within 12 months (starting March 2020). We studied the clinical impact of metformin on vitamin B12 deficiency and stroke evolution. Student's *t*-test and ANOVA were used to compare the groups of patients and to determine whether there was any direct or indirect effect of metformin use on vitamin B12 deficiency and stroke.

Results: In total, 80 patients were admitted with ischemic stroke. Clinical status and biochemical data were collected and compared with healthy volunteers. There were 39 diabetic patients, 16 took metformin for at least 1 year. Among those who took metformin for at least 1 year, 9 had vitamin B12 level < 240 pg/ml (56.2%); 23 diabetic patients did not get metformin and only 4 had vitamin B12 level < 240 pg/ml (17.4%) ($P = 0.014$).

Conclusions: T2DM is a significant risk factor to the development of ischemic stroke. We found an association between metformin use and vitamin B12 deficiency and an association between vitamin B12 deficiency and stroke risk in patients with T2DM. Diabetic patients who are taking metformin should monitor their vitamin B12 level.

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KEY WORDS: metformin, quality of care, type 2 diabetes mellitus (T2DM), vitamin B12 deficiency

Type 2 diabetes mellitus (T2DM) is a known risk factor to the development of cardiovascular disease and stroke, but the impact of diabetes on stroke incidence has yet to be determined. The prevalence of T2DM and its complications increase each year in the Western world, increasing with age and with the Western lifestyle (e.g., more obesity, less physical activity). The Greater Cincinnati/Northern Kentucky Stroke Study found that there are age-, gender-, and race-specific characteristics of diabetic patients with stroke. These patients are younger than 65 years of age, African American, and male. They have other co-morbidities such as hypertension, coronary artery disease, and hyperlipidemia [1]. A meta-analysis of 66 eligible studies found that 28% of patients with stroke had T2DM, with a higher incidence of ischemic stroke (33%) compared with hemorrhagic stroke (26%) [2]. Patients diagnosed with T2DM are at increased risk to develop ischemic stroke, mainly because of cerebral small vessel disease. Acute hyperglycemia and glucose imbalance are the greatest risks associated with poor prognosis of patients with stroke and the post-stroke rehabilitation process [3]. Some risk factors of stroke cannot be changed, such as older age, genetic hereditary disorders, systemic inflammation, infection, air pollution, and cardiac disorders (not only atrial fibrillation). However, there are modifiable risk factors that can be controlled, like smoking, hypertension, and T2DM. It is important to change lifestyle and to control T2DM and hypertension to overcome the non-modifiable risk factors [4].

Recent evidence shows that cerebral microvascular dysfunction could be the mechanistic pathway in patients with T2DM, leading to impaired cognitive status, depression, and stroke [5].

We studied the effect of vitamin B12 deficiency on stroke risk among patients with T2DM. We also sought to determine whether vitamin B12 deficiency was related to metformin.

PATIENTS AND METHODS

We conducted a prospective study of 80 patients with acute ischemic stroke and 30 healthy volunteers from March 2020 until March 2021. Clinical parameters included sex, ethnicity, co-morbidities (T2DM, hypertension, and hyperlipidemia), vitamin B12 levels, and B9 levels [Table 1]. We defined vitamin B12 level below 240 pg/ml as vitamin B12 deficiency. The study was approved by the Helsinki committee of the medical center, and every participant signed a consent form before enrollment. Vitamin B12 was determined using the ARCHITECT B12 assay (a chemiluminescent micro particle intrinsic factor assay for the quantitative determination of vitamin B12 in human serum and plasma on the ARCHITECT system, Abbott Ireland, Diagnostic Division; Lisnamuck, Longford Co. Longford, Ireland).

We used chi-square test for categorical variables and *t*-test for continuous variables to determine differences between the groups.

RESULTS

Patients were older (68 years old vs. 50 years old) ($P < 0.001$). None of our patients died during the 12-month follow-up period, and none developed hemorrhagic stroke. All had a lacunar ischemic stroke, and all were treated with thrombolysis.

The patient group included 57 males (71.2%) and 23 females (28.8%); 34 Arabs (42.5%) and 46 Jews (57.6%). No difference was observed between these groups of patients in relation to vitamin B12 or B9 level, or any other clinical parameters. The volunteer group included 10 males (33.3%) and 20 females (66.7%).

Regarding vitamin B12, 19 patients (23.8%) were diagnosed with a deficiency, 13 of them had T2DM (68.4%); 39 patients had T2DM; 13 diabetic patients had vitamin B12 deficiency (33% of the diabetic patients). Sixteen patients were treated with metformin and 9 of them had vitamin B12 deficiency (56%), compared with 4 of 23 diabetic patients (17.4%) who were not treated with metformin ($P = 0.01$). In total, 66 patients had hypertension (82.5%), 53 had hyperlipidemia (66.2%) [Table 2].

Male patients were relatively younger (66 years old) than females (71 years old) but without a statistical significance ($P = 0.07$). Male patients had higher creatinine level than females (1.10 vs. 0.90, $P = 0.02$), and were taller (168.9 cm vs. 163.3 cm, $P = 0.001$); 33 patients were smokers. Non-smokers were older (70.98 years old vs. 63.91 years old) and were taking statins more often (55% vs. 30%).

No correlation was found between age and vitamin B12 level (Pearson correlation, $P = 0.910$).

Almost half of the stroke patients were taking aspirin and statins before the acute vascular event. However, these medications had no effect on vitamin B12 level, with no statistical difference between the two groups (23.1% of the stroke patients who took aspirin had vitamin B12 deficiency compared to 24.4% who did not take aspirin and had low vitamin B12 level ($P = 1.00$); 27.8% of the stroke patients who took statins had vitamin B12 deficiency as compared to 20.5% who did not take statins, $P = 0.598$).

Table 1. Clinical characteristics of stroke patients and controls

Characteristic	Stroke patients, n=80	Healthy volunteers, n=30	*P*-value
Age, years	68.06 ± 10.47	50.07 ± 7.49	0.001
Sex (females)	23 (28.8%)	20 (66.7%)	0.001
Vitamin B12 (pg/ml)	417 ± 249	437 ± 212	0.514
Vitamin B12 deficiency*	19 (23.8%)	0	0.003
Folic acid (pg/ml)	7.51 ± 4.22	6.15 ± 2.43	0.184
Folic acid deficiency	7 (8.8%)	2 (6.7%)	0.723
Type 2 diabetes mellitus	39 (48.8%)	0	
Hypertension	66 (82.5%)	0	
Hyperlipidemia	53 (66.2%)	0	
Coronary artery disease	28 (35%)	0	
Basal metabolic index	28.41 ± 4.88		
Metformin	16 (39% of the diabetic patients)		
Aspirin	39 (48.8%)		
Statins	36 (45%)		

*Vitamin B12 deficiency was defined as < 240 pg/ml

Table 2. Vitamin B12 level in diabetic patients with stroke

Characteristic	Stroke	T2DM	T2DM + Metformin	T2DM no Metformin
Number of patients	80	39	16	23
Vitamin B12 deficiency	19	13	9	4
Percent	23.8%	33.3%	56%	17.4%

T2DM = type 2 diabetes mellitus

DISCUSSION

Stroke was more prevalent in diabetic patients, especially those with vitamin B12 deficiency (one-third of the diabetic patients). Vitamin B12 deficiency was significantly more prevalent in diabetic patients who were treated with metformin. Overall, 24% of the patients admitted with stroke had vitamin B12 deficiency, most (68%) were diabetic patients.

It is not clear whether the association between T2DM and stroke is related to the complexity of T2DM and its cardiovascular risks; such as, endothelial dysfunction, high level of reactive oxygen species, or systemic inflammation secondary to metformin use per se, and/or due to vitamin B12 deficiency.

A meta-analysis of 54 studies showed that the admission glucose level had a significant impact on the clinical outcome of diabetic patients admitted with stroke, and on the post-thrombolysis clinical outcome [6]. Another study found that even though T2DM is an independent risk factor for the development of ischemic stroke, aggressive treatment of dyslipidemia and hypertension can decrease the risk and the severity of stroke among diabetic patients [7].

Metformin has been linked to the development of stroke in diabetic patients. Diabetic patients on hemodialysis who took metformin had a significantly higher risk of developing ischemic stroke compared to diabetic patients who did not take metformin [8]. Metformin was found to be the only medication that was significantly associated with stroke when other medications were examined (antihypertensive, anti-diabetic, and antiplatelets medications). Metformin causes vitamin B12 deficiency through impaired calcium dependent pathway, blocking vitamin

B12 binding to its intrinsic factor (IF) at the distal ileum. It may also cause changes in small bowel motility with overgrowth of bacteria, leading to impaired absorption of the complex IF-vitamin B12. Another possible mechanistic pathway is altered enterohepatic circulation caused by metformin with accumulation of vitamin B12 in the liver and inhibition of IF secretion by the parietal cells in the gastric mucosa [9].

Vitamin B12 deficiency contributes to myelopathy, peripheral and autonomic neuropathy, subacute combined degeneration of the spinal cord, areflexia, and loss of vibration sensitivity. Poor memory, cognitive impairment (due to dementia), delirium, and depression could be caused by severe vitamin B12 deficiency [10]. The elderly are particularly at risk to develop vitamin B12 deficiency because of dietary habits and the higher rate of atrophic gastritis. It is estimated that 15% of patients older than 65 years old, 23% of octogenarians, and 35% of centenarians have vitamin B12 deficiency [9]. An observational prospective study found that 24.5% of the diabetic patients who took metformin had vitamin B12 levels below 200 pg/ml, and 34.5% of these patients had vitamin B12 levels lower than 300 pg/ml [9]. The 2021 American Diabetes Association Standards of Medical Care recommended measuring vitamin B12 levels periodically in patients who are taking metformin for long periods of time [10]. An association was found between metformin dose and vitamin B12 deficiency. A 1 mg daily increase of metformin was associated with 0.142 pg/ml decrease in vitamin B12 level. However, multivitamins that were taken concurrently protected diabetic patients from the development of vitamin B12 deficiency [10].

The mechanism that may explain the association between vitamin B12 deficiency and stroke could be attributed to the folate cycle and homocysteine. High homocysteine levels have been associated with vascular events and cardiovascular catastrophic events in young adults. Lack of vitamin B12 may lead to inefficient recycling of homocysteine, with its accumulation in the blood. The enzyme methylenetetrahydrofolate reductase (*MTHFR*) is crucial for the folate cycle and for the degradation of homocysteine. Subjects with a heterozygous mutation in the *MTHFR* gene may develop high homocysteine levels if they are not getting enough vitamin B12 supplementation. Lack of vitamin B12 supplementation in these patients leads to high homocysteine levels and to vascular events, including stroke [11]. Low vitamin B12 levels within the normal range (between 150 and 304 pmol/L) have been reported to be associated with poor memory mediated by reduced structural integrity of the hippocampus. This effect was more evident in older patients. Animal and human studies showed that vitamin B12 deficiency caused neuronal degeneration and atrophy of the gray matter associated with regional demyelination and impaired neurogenesis with loss of synaptic contacts [12].

Small vessel disease is quite typical in diabetic patients. The blood–brain barrier dysfunction may play a role in the development of small vessel disease, and the development of ischemic white matter lesions (WML). An experimental animal model has shown that vitamin B12 deficiency caused an increase in myelinolytic tumor necrosis factor and a decrease in interleukin 6 and epidermal growth factor with edema of the blood brain barrier and the periventricular WML [13].

A prospective study examined vegetarians (70% of them had vitamin B12 deficiency) treated with vitamin B12 for 24 weeks. They had a significant improvement in the brachial artery flow medicated dilatation and in their intima media thickness of the carotid arteries. This study may suggest that vitamin B12 deficiency affects the systemic arterial vascular bed, and that it can be reversible once vitamin B12 is supplemented [14].

It was found also that low to borderline levels of vitamin B12 could be related to cognitive impairment, especially in the elderly [15], and that intravenous vitamin B1 supplementation given to a partially paralyzed patient (a patient who underwent gastric bypass surgery a year earlier) caused full recovery [16].

LIMITATIONS OF THE STUDY

Due to the coronavirus disease 2019 (COVID-19) pandemic, we could not match the control group to our patients because many patients were afraid to enter the medical center during that time to participate in a clinical study at the hospital. Thus, the patient group was small. We did not consider other anti-diabetic medications because we were focused on metformin only, due to the possible effect on vitamin B12 levels and the known correlation with stroke. It was important for us to examine whether the stroke incidence was related to metformin or to vitamin B12 deficiency that could be caused by metformin.

CONCLUSIONS

Vitamin B12 deficiency was more prevalent among diabetic patients, mainly in those who were treated with metformin for more than 1 year. Diabetic patients, mainly older individuals and especially those who are treated with metformin, are at increased risk to develop vitamin B12 deficiency. Vitamin B12 deficiency may affect the cognitive function and the cerebrovascular small blood vessels as well as the big blood vessels. Both microvascular disease and macrovascular disease cause damage to the brain leading to stroke and vascular dementia. Vitamin B12 level should be monitored closely in patients with T2DM, especially those treated with metformin, to prevent the development of stroke.

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We are not the same persons this year as last; nor are those we love.
It is a happy chance if we, changing, continue to love a changed person.
William Somerset Maugham (1874–1965), English writer, known for his plays, novels, and short stories

Capsule

Limiting lymphopenia after glioblastoma

Lymphopenia, a lack of white blood cells, frequently occurs in patients with glioblastoma (GBM) after chemoradiation therapy, and its association with worse survival is not well understood. Analyzing peripheral blood from 20 patients treated for GBM, **Ghosh** and co-authors identified an elevated concentration of myeloid-derived suppressor cells (MDSCs) in patients with lymphopenia after treatment. The authors developed murine models to demonstrate a causal relationship between radiation-induced MDSCs and lymphopenia. Pharmacologic inhibition of MDSCs abrogated lymphopenia and improved survival in mice, suggesting that these classes of drugs may be a potential treatment to reduce lymphopenia in patients with GBM.

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Capsule

Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer

Despite standard-of-care treatment, more than 30% of patients with resectable colorectal cancer (CRC) relapse. Circulating tumor DNA (ctDNA) analysis may enable postsurgical risk stratification and adjuvant chemotherapy (ACT) treatment decision making. **Kotani** and colleagues reported results from GALAXY, which is an observational arm of the ongoing CIRCULATE-Japan study (UMIN000039205) that analyzed presurgical and postsurgical ctDNA in patients with stage II–IV resectable CRC (n=1039). In this cohort, with a median follow-up of 16.74 months (range 0.49–24.83 months), postsurgical ctDNA positivity (at 4 weeks after surgery) was associated with higher recurrence risk (hazard ratio [HR] 10.0, $P < 0.0001$) and was the most significant prognostic factor associated with recurrence risk in patients with stage II or III CRC (HR 10.82, $P < 0.001$). Furthermore, postsurgical ctDNA positivity identified patients with stage II or III CRC who derived benefit from ACT (HR 6.59, $P < 0.0001$). The results of this study support the use of ctDNA testing to identify patients who are at increased risk of recurrence and are likely to benefit from ACT.

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