THE RISK OF COBALAMIN DEFICIENCY SYMPTOMS RELATED TO LONG-TERM METFORMIN USE IN T2DM PATIENTS

Y.D. Hendrawati^{1,2,*}, R. Andrajati¹, S. Supardi³, A. Ariyani⁴

¹Universitas Indonesia, Faculty of Pharmacy, Depok, ²PT Dixa Medica, Titan Center, Tangerang Selatan,

³Litbangkes, ⁴Pasar Rebo General Hospital, Clinical Pathology, Jakarta, Indonesia

Abstract

Background. Metformin is the first-line oral antidiabetic agent used in the treatment of diabetes mellitus. One of the adverse reactions of the long term use of metformin is cobalamin malabsorption. Clinical and laboratory findings are important in the diagnosis of cobalamin deficiency.

Objective. This study aimed to evaluate the risk of cobalamin deficiency symptoms related to long-term use of metformin in type 2 diabetes mellitus patients at Pasar Rebo General Hospital in Jakarta.

Setting. This quantitative, observational study with retrospective cohort design was conducted in outpatient department Pasar Rebo General Hospital November 2015 until January 2016.

Methods. 200 subjects were recruited and divided into two groups, patients who had been taking metformin for 1-3 years and patients who had been taking metformin for more than 3 years. Each patient was assessed for the presence of cobalamin deficiency symptoms.

Main outcome measure. Cobalamin deficiency symptoms evaluated were symptoms of neuropathy (measured by DN4 questionnaire) and hematologic abnormalities associated to cobalamin deficiency, i.e. macrocytic erythrocyte, hypersegmented neutrophils, and giant bands.

Results. There are significant differences in the proportions of neuropathy symptoms (RR 2.36, 95%, p=0.000) and hematologic abnormalities (RR 1.5, 95%, p=0.007) between the two groups.

Conclusions. Long-term use of metformin (≥ 3 years) may increase the risk of cobalamin deficiency symptoms in type 2 diabetes mellitus patients.

Key words: metformin, cobalamin deficiency, neuropathy, vitamin B12, macrocytic erythrocyte.

INTRODUCTION

Diabetes mellitus is one of the most common chronic diseases in the world, and the prevalence

of diabetes in adults has been increasing in the past decades (1). According to International Diabetes Federation (2013), Indonesia is the seventh country in the number of people with diabetes worldwide (2).

Metformin is the first-line oral antidiabetic agent recommended in the treatment of type 2 diabetes mellitus (3). There are few adverse effects related to the use of metformin, as it induces cobalamin malabsorption, which may increase the risk of developing cobalamin deficiency. The mechanism of metformin-induced cobalamin deficiency is supposed to be through disruption of ileal calcium-dependent membrane involved in cobalamin absorption (4). Some studies show that up to 30% percent of diabetic patients had metabolically confirmed cobalamin deficiency. Patients on metformin also had low serum cobalamin levels compared to those who do not use metformin (5-7).

Vitamin B12 (cobalamin) is a water-soluble vitamin that plays an important role in DNA synthesis and neurological processes. Cobalamin deficiency can lead to a wide spectrum of hematologic and neuropsychiatric abnormalities that need a suitable treatment. Traditionally, diagnosis of cobalamin deficiency is carried out by measurement of serum cobalamin level, along with clinical examination, nevertheless, approximately 50% patients have normal serum cobalamin level while having clinical symptoms or low cobalamin metabolite levels (8).

Cobalamin is a cofactor for only two enzymes: methionine synthase and L-methyl-malonyl-coenzyme A mutase. Vitamin B12 interacts with folate and is responsible for the case of megaloblastic anemia seen in both vitamin deficiencies (9). The methylation of homocysteine to methionine requires both 5-methyltetrahydrofolate (methyl-THF) as methyl donor and methylcobalamin as co-enzyme. This reaction is the first step in the pathway by which

*Correspondence to: Yosephine Dian Hendrawati, Fakultas Farmasi Universitas Indonesia, Kampus UI Depok, Gedung A Rumpun Ilmu Kesehatan Lantai 3, Depok, Jawa Barat, 16424, Indonesia, E-mail: maria.yosephinedian@gmail.com

methyl-THF is converted into all the intracellular folate co-enzymes after entering bone marrow and other cells from plasma. In cobalamin deficiency, methyl-THF accumulates in the plasma, while intracellular folate concentrations fall due to failure of formation of intracellular folate polyglutamates because of lack of THF. This explains the abnormalities of folate metabolism following cobalamin deficiency. In deficiencies of either folate or cobalamin there is a failure to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTTP). The coenzyme 5,10-methylene tetrahydrofolate polyglutamate is needed for this reaction and the availability of this co-enzyme is reduced in either deficiency. Dyssynchrony between the maturation of cytoplasm and that of nuclei leads to some hematologic abnormalities. Oval macrocytes, usually with considerable anisocytosis and poikilocytosis, are the main feature. The MCV is usually more than 100 fL unless a cause of microcytosis (e.g. iron deficiency or thalassaemia trait) is present. Some of the neutrophils are hypersegmented (more than five nuclear lobes). Anemia does not always occur, but there may be leucopenia due to a reduction in granulocytes and lymphocytes (10).

As a cofactor of methionine synthase in the synthesis of methionine, cobalamin also plays an important role in the production of the precursor of the universal methyl donor S-adenosylmethionine (SAM), which is involved in different epigenomic regulatory mechanisms and especially in the brain development. A cobalamin deficiency expresses itself by a wide variety of neurological manifestations such as paraesthesias, skin numbness, coordination disorders and reduced nerve conduction velocity (11).

Although serum cobalamin measurement currently remains the first-line test in diagnosing cobalamin deficiency, clinical picture is the most important factor in assessing the significance of the test result assessing cobalamin status because there is no gold standard test to define deficiency. In the presence of discordance between the test result and strong clinical features of cobalamin deficiency, treatment should be started as soon as possible to avoid further neurological impairment. If a patient is found with strong clinical features of cobalamin deficiency, but serum cobalamin level lies within the reference range (false normal), other tests may be used to determine an underlying functional or biochemical deficiency. These tests, plasma homocysteine, plasma methylmalonic acid, and serum holotranscobalamin, may help, but these tests are

not widely available in all laboratories. Peripheral blood smear examination can help in determining cobalamin deficiency-associated conditions. Identification of oval macrocytes, hypersegmented neutrophils, and circulating megaloblasts in the blood film are the typical features of cobalamin deficiency. Elevated mean corpuscular volume (MCV) (i.e. >100 fL) is also a sign, but it is not a specific indicator of cobalamin deficiency, thus the absence of raised MCV cannot be used to exclude the need of cobalamin testing because neurological impairment occurs with a normal MCV in 25% of cases (12).

Average body stores of cobalamin is huge relative to estimated daily loss or requirement, thus cobalamin deficiency reflects a chronic process and typically takes years to develop in adults. Cobalamin is also tightly chaperoned everywhere by specific binding proteins and receptors that regulate how much traverses gut and other cell membranes, how much is reabsorbed after biliary secretion, and how much is retained by the body. As long as specific absorption via IF remains intact, transport and distribution are usually maintained. When there is absence of intrinsic factor (IF) or ileal uptake fails, absorption of cobalamin fails. As the malabsorption persists, deficiency progresses, and clinical features may appear within 2-5 years (13). A cross-sectional cohort study showed that patients exposed to metformin for more than one year had lower cobalamin level compared to the non-exposed group (14). A nested case-control study also showed that among those using metformin for 3 years or more, the adjusted odds ratio of developing cobalamin deficiency was 2.39 compared with those receiving metformin for less than 3 years (15).

Annual outpatient report of Pasar Rebo General Hospital in Jakarta 2014 shows that type 2 diabetes mellitus is the highest number of cases (15,380 cases) in the year. The risk of cobalamin deficiency associated with the use of metformin in this hospital has not been studied.

This study aimed to evaluate the risk of long-term use of metformin in the development of cobalamin deficiency symptoms in type 2 diabetes mellitus patients at Pasar Rebo General Hospital in Jakarta. The aim of this study was not to diagnose whether there is cobalamin deficiency due to long-term metformin use, but we tried to find any approach to detect the symptoms or signs of cobalamin depletion or cobalamin deficiency due to long-term use of metformin. The haematological abnormalities and neurological disorders were not specific for cobalamin

deficiency, but those parameter could be very helpful for the early detection of cobalamin deficiency in patients receiving metformin in our country.

MATERIALS AND METHOD

This is a quantitative, observational study with retrospective cohort design. This study was conducted in the outpatient department of Pasar Rebo General Hospital since November 2015 until January 2016.

The patients enrolled in this study are registered in national health care system, aimed to ensure affordability of health care for all people in the country. The glycemic and renal function were examined according to physician's recommendation for each patient. This protocol was approved by Research Ethics Committee, Faculty of Medicine, Universitas Indonesia. Researchers collaborated with the physicians in Pasar Rebo General Hospital in the recruitment step to verify the patients about their diagnosis and medications. Then patients were asked about their willingness to participate in the study, and we added the examination of peripheral blood smear and complete blood count to their laboratory referral.

Patients were approached to enrol into the trial and gave written informed consent. Patients divided into two groups, each including 100 cases, patients who had been taking metformin for 1-3 years (group I) and patients who had been taking metformin for more than 3 years (group II). Patients with known anemia (possibly due to the lack of vitamin B12 and folic acid), vegetarian diet, and long term use of non-steroid anti-inflammarory drugs (NSAIDs) were excluded. During patients' routine visit to internal medicine department, medical history was obtained, laboratory investigation and assessment of neuropathy were performed. Cobalamin deficiency symptoms

evaluated were symptoms of neuropathy (measured by DN4 questionnaire (16) and hematologic abnormalities associated to cobalamin deficiency, i.e. macrocytic erythrocyte, hypersegmented neutrophils, and giant bands. Score DN4 ≥ 4 is considered neuropathy. Relative risk of neuropathy symptoms and hematologic abnormalities related to cobalamin deficiency were calculated. Some confounding variables that are considered also affect serum cobalamin level were also assessed, such as age, body mass index (BMI), and therapy with proton pump inhibitors (17-20). Data were analyzed statistically using Chi-Square test with confidence level 95%.

RESULTS

We identified 200 eligible patients. Table 1 shows baseline characteristics of all patients analyzed. Patients with history of metformin use more than 3 years (group II) were older than those with history of metformin use between 1-3 years (group I). The other characteristics were comparable between the two groups.

Ninety two patients (46%) were found overweight (BMI ≥ 25 kg/m²) and 108 patients (54%) have normal BMI. The average BMI of the patients was 25.27 kg/m² (overweight). The age of patients enrolled in this study lies between 37-86 years with average of 60.18. Fifty five percent of the patients were elderly (≥ 60). Sixty one (30%) patients were in the middle of proton pump inhibitor therapy.

Table 2 shows the incidences of cobalamin deficiency symptoms in patients receiving metformin. The incidences of neuropathy symptoms based on DN4 measurement and hematologic abnormalities related to cobalamin deficiency were significantly higher in the group of metformin use of more than 3 years. Sixty

Table 1. Baseline characteristics of patients receiving metformin

	Group I (metformin 1-3 years) n=100	Group II (metformin >3 years) n=100	p
Sex			
Male	29	36	0.291
Female	71	64	
Age			
<60 years	53	36	0.023
≥ 60 years	47	64	
Body Mass Index			
<25 kg/m ²	50	42	0.321
≥ 25 kg/m ²	50	58	
Therapy with PPI			
Yes	30	31	1.000
No	70	69	

Table 2. Incidences of cobalamin deficiency symptoms in patients receiving metformin in Pasar Rebo General Hospital

Cobalamin deficiency parameters	Group I (metformin 1-3 years) n=100	Group II (metformin >3 years) n=100	p
Neuropathy symptoms			
• Yes (DN4 score $\geq$ 4)	28	69	0.001
• No (DN4 score<4)	72	31	
Hematologic abnormalities related to cobalamin deficiency			
• Yes	39	59	0.007
• No	61	41	

nine (69%) patients in group II had DN4 score $\geq$ 4, thus considered to have neuropathy. Only twenty eight (28%) patients in group I had DN4 score $\geq$ 4 (RR=2.36; p=0.000; CI 95%). Fifty nine (59%) patients in group II had hematologic abnormalities related to cobalamin deficiency. Only thirty nine (39%) patients in group I had hematologic abnormalities related to cobalamin deficiency (RR=1.5; p=0.007; CI 95%).

Multivariate analysis of confounding variables using logistic regression found that therapy with proton pump inhibitor can affect cobalamin status causing neuropathy symptoms with odds ratio (OR) 4.367. Meanwhile, age can affect cobalamin status causing hematologic abnormalities with odds ratio (OR) 1.671. Our study on the risk of cobalamin deficiency symptoms related to long-term use of metformin had three main findings. First, it can be seen that patients with longer duration of metformin use were found with a greater number of cobalamin deficiency symptoms. Second, cobalamin deficiency related to metformin use has not been fully concerned by physician so early and routine screening of this condition in patients using metformin has not been performed. Third, clinical and laboratory examination of cobalamin deficiency-associated conditions such as macrocytic anemia and symptoms of neuropathy could be very helpful in early detection of metformin's adverse reaction, especially when serum cobalamin measurement is not routinely performed due to economical reason in some developing countries.

DISCUSSION

The finding of decreases in cobalamin concentration during metformin treatment is not novel and has been reported before. However, the clinical significance due to this condition was not always assessed. As stated above, cobalamin deficiency is the common cause of macrocytic anemia and wide spectrum of neuropsychiatric disorders. Besides, there is no gold standard yet in the diagnosis of cobalamin deficiency since serum cobalamin could show a false

normal result.

Metformin is thought to induce malabsorption of cobalamin and intrinsic factor complex by disruption of calcium-dependent membrane in the ileum, an effect reversed with supplemental calcium (4). Study in rats showed that metformin treatment increases liver accumulation of B12, thereby resulting in decreases in circulating B12 and kidney accumulation of the vitamin (21). The clinically important consequences of the decrease in cobalamin concentrations are macrocytic anemia and neuropathy (6).

Our result showed that patients with longer use of metformin tend to show more cobalamin deficiency-related hematologic abnormalities. A cross-sectional study found that 27% patients on metformin were anaemic and 2 of them had macrocytosis (7). The most frequent result in our study was hypersegmented neutrophil. Macrocytosis was found in 4 patients from group II. There was no subject found with MCV >100 fL, presumably due to the high incidence of iron deficiency in diabetic patients.

The neurologic manifestations begin pathologically with demyelination, followed by axonal degeneration and axonal death that can cause eventual irreversible damage. The spinal cord, brain, optic nerves, and peripheral nerves may all be affected by cobalamin deficiency (22). Wile and Toth (23) found that beside low cobalamin level, metformin-treated patients also had an elevated level of homocysteine and methylmalonic acid. They also identified worsening peripheral neuropathy in those patients. The early clinical manifestations of cobalamin deficiency are numbness and paresthesia in the feet, and later followed by muscle weakness, ataxia, sphincter disturbance, and changes in mental status. Neurological finding of cobalamin deficiency is often misdiagnosed with diabetic peripheral neuropathy, but in some point they can be determined, for example by assessing the presence of reflexes. Reflexes are usually preserved in the case of cobalamin deficiency, but almost lost in the case of diabetic neuropathy (24). In our study, the frequent symptoms found were numbness and

tingling sensation.

Regular measurement of cobalamin concentration is suggested in patients taking long term therapy with metformin (6). De Jager *et al.* used prospective cohort design to assess the risk of cobalamin deficiency. They used 2250 mg metformin in three divided doses for 4.3 years. The absolute risk of cobalamin deficiency at study end was 7.2% in the metformin group. Our study used a retrospective cohort. Metformin dose among the patients varies between 500-1500 mg daily.

Patients with short term use of metformin (<1 year) were not included in this study since cobalamin deficiency symptoms in those patients might be confused with other causes. A cross-sectional cohort study also used similar inclusion criteria, i.e. patients exposed to metformin for more than one year. They found that long-term exposure of metformin carries potential risk of cobalamin deficiency. It could be seen from 26.7% incidence of low cobalamin serum, 21.6% low holotranscobalamin, and 9.7% higher serum homocysteine level, although they did not assess the clinical significance of these results (14). The effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 had been studied. Low cobalamin level was found in 14% patients in the group after 16 weeks therapy with metformin 850 mg three times daily, but the clinical significance remained to be investigated (25).

Duration of metformin use is suspected as an important factor in the development of cobalamin deficiency in patients taking metformin. In the study about identification of risk factors for metformin related cobalamin deficiency, it was found that among those using metformin for three years or more, the odds ratio of cobalamin deficiency is larger compared with those receiving metformin for less than 3 years (26). A retrospective case control study conducted in Korea found that cobalamin deficient patients had a longer duration of metformin use and a higher daily metformin dose. Metformin duration was differentiated as <4 years, 4-10 years, and ≥ 10 years, and the metformin dose was differentiated as ≤ 1000 mg, 500-2000 mg, and ≥ 2000 mg. The prevalence of cobalamin deficiency in metformin-treated type 2 diabetes patients was 9.5%. Clinical significance of this finding was not assessed such as symptoms of neuropathy or megaloblastic anemia. MCV was examined, but the mean result was still within the normal range (27). It fits the theory that MCV alone cannot be used to predict cobalamin deficient condition since there are many possibilities

of other low MCV causes such as iron deficiency. Low cobalamin level causes high MCV, but low iron level causes low MCV. In patients with both conditions, low iron and low cobalamin level, the MCV is usually in the normal range. That is why we did not use MCV as the variable, but the result of blood smear instead. Blood smear will show better the real condition of blood cells so we can see if cobalamin deficiency might happen.

Our analysis on confounding variables found that age and the use of proton pump inhibitor agent can also affect vitamin B12 status in diabetic patients. A cross-sectional study found that metformin therapy in elderly associated with cobalamin deficiency. Data were recorded from patients aged >60 years. This population has higher risk of cobalamin deficiency, e.g. due to physiological changes, pathologic conditions, or medications used. Geriatric patients with metformin use have a significantly lower cobalamin serum level than those who do not use metformin. The different result with our study is there was no significant difference of cobalamin level between long-term (>3 years) and short-term users (28).

Acid-suppressive agents such as histamin-2 receptor antagonist and proton pump inhibitor are also associated in the development of cobalamin deficiency. 40.7% diabetic patients also experienced gastroesophageal reflux disease, thus the concomitant use of metformin and acid-suppressive agents might be found in clinical practices (29). Via various mechanisms, concomitant use of metformin and acid-suppressing medications can contribute to neuropathy among individuals with diabetes. Regarding this possibility, health care providers and people with diabetes also should be aware of the many strategies and alternatives that have been shown to improve cobalamin deficiency and associated neuropathy (19). Long, Atwell, Yoo, and Solomon (30) wrote that proton pump inhibitors and metformin alone were not associated with a significant difference in cobalamin deficiency, but the combination was associated with a significant cobalamin deficiency.

A limitation of this study is that we did not measure cobalamin or other metabolites level in each patient. Serum cobalamin measurement is not covered by health insurance in Indonesia and is still considered as an expensive examination. Therefore we only conclude that the use of long term metformin increased the risk of cobalamin deficiency symptoms in type-2 diabetic patients. Patients with positive symptoms were advised about further checking of cobalamin status so appropriate treatment could be given.

In conclusion, finding a prevalence of 69% neuropathy symptoms and 59% hematologic abnormalities related to cobalamin deficiency in patients receiving metformin for more than 3 years, we therefore recommend the routine screening of cobalamin status in patients treated with metformin. Early detection of cobalamin deficiency allows health providers to use many strategies in order to prevent worsening clinical manifestations and improve patients' quality of life. If routine cobalamin serum level testing is not affordable for most patients such as in Indonesia, a preventive strategy by giving vitamin B12 with suitable dose in metformin treated patients may be considered.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors acknowledge the funding of PT Dexa Medica, also the help from nurses and laboratory staffs in Pasar Rebo General Hospital.

References

- Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice* 2010; 87(1):4-14.
- Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice* 2014; 103(2):137-149.
- American Diabetes Association. Approaches to glycemic treatment. *Diabetes Care* 2018;41(Suppl. 1):S73-S85.
- Bauman WA, Shaw S, Jayatilake E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. *Diabetes Care* 2000; 23(9):1227-1231.
- Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross-sectional study. *J Am Board Fam Med* 2009; 22(5):529-534.
- de Jager J, Kooy A, Lehert P, Wulfel MG, van der Kolk J, Bets D, Verburg J, Donker AJ, Stehouwer CD. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomized placebo controlled trial. *BMJ* 2010; 340:c2181.
- Marar O, Senturk S, Agha A, Thompson C, Smith D. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin. *Royal College of Surgeons in Ireland Student Medical Journal* 2011; 4(1):16-20.
- Oh RC, Brown DL. Vitamin B12 deficiency. *Am Fam Physician* 2003; 67(5):979-986.
- Stabler SP. Vitamin B12 deficiency. *N Engl J Med* 2013; 368:149-160.
- Hoffbrand AV, Higgs DR, Keeling DM, Mehta AB. *Postgraduate Haematology*. 7th ed. New York: John Wiley & Sons; 2016:53-71.
- Grober U, Kisters K, Schmidt J. Neuroenhancement with vitamin B12—underestimated neurological significance. *Nutrients* 2013; 5(12):5031-5045.
- Devalia V, Hamilton MS, Molloy AM. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *British Journal of Haematology* 2014; 166(4):496-513.
- Carmel R. How I treat cobalamin (vitamin B12) deficiency. *Blood* 2008; 112(6):2214-2221.
- Hermann LS, Nilsson B, Wettre S. Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study. *Br J Diabetes Vasc Dis* 2004; 4(6):401-406.
- Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Intern Med* 2006; 166(18):1975-1979.
- Bouhassira D, Attal N, Alchaar H, Boureau F, Brochet B, Bruxelle J, Cunin G, Fermanian J, Ginies P, Grun-Overdyking A, Jafari-Schluep H, Lantri-Minet M, Laurent B, Mick G, Serrie A, Valade D, Vicaud E. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* 2005; 114(1-2):29-36.
- Loikas S, Koskinen P, Irjala K, Loppinen M, Isoaho R, Kivela S, Pelliniemi T. Vitamin B12 deficiency in the aged: a population-based study. *Age Ageing* 2007; 36 (2):177-183.
- Baltaci D, Kutlucan A, Turker Y, Yilmaz A, Karacam S, Deler H, Ucgun T, Kara IH. Association of vitamin B12 with obesity, overweight, insulin resistance and metabolic syndrome, and body fat composition; primary care-based study. *Med Glas (Zenica)* 2013; 10(2):203-210.
- Zdilla MJ. Metformin with either histamine H2-receptor antagonists or proton pump inhibitors: A polypharmacy recipe for neuropathy via vitamin B12 depletion. *Clinical Diabetes Journal* 2015; 33(2):91-95.
- Lam JR, Schneider JL, Zhao W, Corley DA. Proton pump inhibitor and histamine 2 receptor antagonist use and vitamin B12 deficiency. *JAMA*. 2013; 310(22):2435-2442.
- Greibel E, Miller JW, Foutouhi SH, Green R, Nex E. Metformin increases liver accumulation of vitamin B12: an experimental study in rats. *Biochimie* 2013; 95(5):1062-1065.
- Miller A, Korem M, Almog R, Galboiz Y. Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. *J Neurol Sci*. 2005; 233(1-2):93-97.
- Wile DJ, Toth C. Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. *Diabetes Care* 2010; 33(1):156-161.
- Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. *South Med J* 2010; 103(3):265-267.
- Wulfel MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, Donker AJ, Stehouwer CD. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *Journal of Internal Medicine* 2003; 254(5):455-463.
- Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Intern Med* 2006; 166:1975-1979.
- Ko S, Ko S, Ahn Y, Song KH, Han KD, Park YM, Ko SH, Kim HS. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. *J Korean Med Sci* 2014; 29(7):965-972.
- Liu KW, Dai DLK, Ho W, Lau E, Woo J. Metformin-associated vitamin B12 deficiency in the elderly. *Asian J Gerontol Geriatr* 2011; 6(2):82-87.
- Wang X, Pitchumoni CS, Chanraran K, Shah N. Increased prevalence of symptoms of gastroesophageal reflux diseases in type 2 diabetics with neuropathy. *World J Gastroenterol* 2008; 14(5):709-712.
- Long AN, Atwell CL, Yoo W, Solomon SS. Vitamin B12 deficiency associated with concomitant metformin and proton pump inhibitor use. *Diabetes Care* 2012; 35(12):e84-e84.