Journal of
Family Medicine and Disease Prevention

REVIEW ARTICLE

Vitamin B12 for Diabetes Patients Treated with Metformin

Roman Pawlak*

Department of Nutrition Science, East Carolina University, USA

*Corresponding author: *Roman Pawlak, PhD, Department of Nutrition Science, East Carolina University, Greenville, USA, Tel: 252-744-1013, E-mail: pawlakr@ecu.edu*

Abstract

Metformin treatment, both duration and dose, is associated with increased risk of vitamin B12 (B12) deficiency. B12 deficiency causes Hyperhomocysteinemia (HHcy), which is associated with an increased risk of a variety of diabetic co-morbidities. As a result, the newest "Standards of Medical Care in Diabetes - 2017", issued by the American Diabetes Association included a recommendation to periodically assess B12 status and, as needed, utilize B12 replacement therapy among diabetes patients receiving metformin. Routine screening, using appropriate B12 assessment methods and interpretation, would enable providers to identify individuals with low B12 levels at an early stage. Prompt treatment with B12 injections or oral supplements may help in reducing the risk of low B12- and HHcy-related sequelae and their associate medical cost. The assessment of B12 status and interpretation of results is not straightforward since several B12 assessment measures are available, including serum/plasma B12, Mean Corpuscular Volume, Homocysteine, Holotranscobalamin II and Methylmalonic Acid. The goal of this manuscript is to 1) Describe available B12 assessment methods including their advantages and disadvantages, 2) Interpret laboratory results of B12 biomarkers, and 3) Discuss available B12 deficiency treatment options. Routine screening, when appropriate B12 status analyses are performed and interpreted, would enable physicians to identify individuals with low B12 at early stage. Treatment with B12 injections or supplements would help in reducing risk of low B12 and HHcy-related symptoms. Such practice would reduce pain and suffering of diabetic patients. It would also decrease medical cost associated with treatments of diabetic co-morbidities.

Introduction

According to the recommendation issued jointly by the American Diabetes Association and the European Association for the Study of Diabetes, concurrently with lifestyle intervention, metformin therapy should be initiated at the time of diabetes diagnoses [1]. Metformin is also recommended for individuals with impaired fasting glucose and/or impaired glucose tolerance who also meet any of the following criteria: are < 60-years-old, have BMI ≥ 35 kg/m², have a family history of diabetes in the first degree of relatives, have elevated triglycerides, have reduced HDL cholesterol, have hypertension, and have hemoglobin A1c > 6.0% [2].

Although metformin is effective in blood glucose control its use is associated with reduced vitamin B12 (B12) concentrations. Results of a recent meta-analysis based on 29 studies with 8,089 participants showed that patients receiving metformin therapy had 2.45 (95% CI 1.74-3.44, p < 0.0001) times higher odds of developing B12 deficiency in comparison to the non-metformin users [3]. There was a mean of 65.8 pmol/L (95% CI 53.6-78.1, p < 0.0001) reduction in the serum B12 level among patients receiving metformin compared to those not receiving metformin treatment. The above results are consistent with the findings published in a systemic review of the impact of metformin on B12 status by Liu, et al. [4]. They found that patients receiving metformin had statistically significantly lower serum B12 concentrations, compared to patients receiving placebo and/or Rosiglitazone (mean difference = 53.93 pmol/L, 95% CI 26.42-81.44, p = 0.0001). The association between metformin use and serum B12 reduction was dose-dependent [4].

Based on available evidence of the association between metformin use and reduced B12 levels, the newly published American Diabetes Association's "Standards of Medical Care in Diabetes - 2017" included a recommendation to periodically assess B12 status and,

Pawlak. J Fam Med Dis Prev 2017, 3:057

• Page 1 of 7 •

when clinically indicated, utilize B12 supplementation among diabetes patients receiving metformin [5]. No guidelines regarding available B12 assessment methods were included in the above-mentioned document. Similarly, no guidelines regarding interpretation of B12 laboratory findings or on available treatment options were outlined.

B12 deficiency results in Hyperhomocysteinemia (HHcy), which is associated with an increased risk of several diabetic co-morbidities. Results of recent meta-analysis, based on 31 studies with a total of 6,394 patients, showed type 2 diabetic patients with HHcy having a 93% higher risk of developing retinopathy (OR = 1.93, 95% CI 1.46-2.53) [6]. Similarly, patients with type 1 diabetes with HHcy had an elevated risk (OR = 1.83, 95% CI 1.28-2.62) [6]. Homocysteine (Hcy) concentration was found to be statistically significantly associated with macular edema among Chinese type 2 diabetes patents [7]. Patients with Diabetic Macular Edema (DME) had a higher Hcy concentration, mean Hcy = 11.4 µmol/L, compared to macular edema - free controls, mean concentration = 8.5 µmol/l, p = 0.000. The association between Hcy concentration and DME was independent of other risk factors, OR = 1.63, CI 1.02-2.14, p = 0.018. Each increase of 5.0 µmol/L in plasma Hcy concentration was associated with a 64% higher risk of DME (OR = 1.64, CI 1.04-2.16, p = 0.019) after controlling for other factors [7].

Assessment of B12 status and interpretation of results is not very straightforward. Several B12 assessment methods are available. These assessment methods have different specificity and accuracy. Some of these assessment types may not be very useful in assessing B12 status. Thus, the goal of this manuscript is to 1) Describe available to clinicians B12 assessment methods, 2) Interpret laboratory results, and 3) Discuss available B12 deficiency treatment options.

B12 assessment techniques and interpretation of laboratory results

Several B12 assessment methods are available for providers. They include serum/plasma B12, Mean Corpuscular Volume (MCV), Homocysteine (Hcy), Holotranscobalamin II (holoTCII) and serum and urinary Methylmalonic Acid (MMA). (Table 1) includes the list of biochemical B12 assessments along with the traditional and evidence-based deficiency cutoffs. Serum B12 along with MCV seem to be used the most in clinical settings. Unfortunately, these two assessment methods are not always accurate in making diagnoses of B12 status.

Serum/plasma B12

Serum B12 is a function of at least two different B12 carriers: holoTCII and Haptocorrin. Haptocorrin carries B12 that has been stored in the liver, whereas holoTCII transports B12 provided via the small intestines. At any given time, at least 80% of circulating B12 is carried via Haptocorrin [8]. The physiological role of Haptocorrin is not clear. It is holoTCII that supplies B12 to cells, including neurologic cells. Individuals with low B12 intake, such as vegans, and those with B12 Malabsorption, which include patients receiving metformin therapy, may have holoTCII so low that serum B12 might solely reflect B12 attached to Haptocorin. For these individuals assessment of serum B12 will poorly reflect the functional B12 status, since even individuals with relatively high B12 values may have functional B12 deficiency reflective in low holo TCII.

Another potential issue with relying on serum B12 values to assess B12 status is that, according to Obeid, et al. B12 may be trapped in plasma in cases with high glucose concentration [9]. This would mean that diabetes patients with poor glycemic control may be at high risk of having elevated B12, while in the same time having functional B12 deficiency. Thus, relying on serum/plasma B12 may yield false negative results for patients with poor glycemic control. Many lab results use serum B12 < 148 pmol/L (< 200 pg/ml, < 200 ng/L) as cutoff for deficiency status. Recent studies showed that this cutoff is insufficient since symptoms of B12 deficiency are often seen among patients with considerably higher serum/plasma B12 [10]. Herbert suggested that a level of 300 pg/ml or less (< 227 pmol/L) should be considered deficient [11]. Herbert's suggestion is supported by other authors, some of whom suggested even higher cutoff criteria, as noted in the following quote: "Levels < 200 ng/L (< 150 pmol/L) are sure signs of a B12 deficiency; but a functional B12 deficiency may also be present at levels under 450 ng/L (~332 pmol/L - author). Persons with B12 concentrations within the reference range may already manifest clinical signs of a Vitamin B12 deficiency. Characteristic examples are cases of clinically and MRI - confirmed severe funicular myelo-

Table 1: Traditional and evidence-based vitamin B12 deficiency reference values.

Assessment	Traditional assessment cutoffs	Evidence-based assessment cutoffs
Serum B12	< 148 pmol/L (< 200 pg/ml)	< 300 pmol/L (< 405 pg/ml)
Homocysteine	> 15 µmol/L	≥ 10 µmol/L
Holo TC II	< 35 pmol/L	< 50 pmol/L
Serum MMA	> 260 nmol/L or > 271 nmol/L	> 260 nmol/L or > 271 nmol/L
Urinary MMA	> 4.3 µmol/L	> 4.0 µmol/L
Urinary MMA/creatinine	> 4.8 mmol/L creatinine	> 3.5 mmol/L creatinine
MCV	> 98 µm³/cell	> 98 µm³/cell

sis despite normal Vitamin B12 levels and prompt response to B12 substitution" [12]. Similarly, according to Schwarz, et al. "If total B12 is applied to identify B12 deficiency, the cutoff values should be elevated to 304 (B12-CLIA) and 368 ng/L (B12-MTP) to improve the predictive power" [13]. In fact, more sensitive assessment methods of B12 status, described below, showed values indicative of a biochemical deficiency among individuals whose serum vitamin B12 concentration was as high as about 400 pmol/L (540 pg/ml) [14]. Clinical responses to neuropathy symptoms have been observed among elderly diabetes patients with serum B12 > 400 pg/ml (295 pmol/L) [15]. Smith and Refsum suggested that physicians should treat patients with serum B12 around 300 pmol/l who show symptoms of a deficiency [16]. Symptoms of B12 deficiency are listed in (Table 2).

Mean Corpuscular Volume

MCV is an assessment of erythrocytes volume [17]. Theoretically, B12 deficiency should result in megaloblastic (macrocytic) anemia, which should be manifested in elevated MCV. However, MCV is also affected by intake and status of folate and iron. In case of high folate intake, megaloblastic anemia may not develop even in cases with severely low B12. Also, iron deficiency results in microcytic anemia. Thus, if a patient has both B12 and iron deficiency, MCV may be shown to be normal, since the impact of B12 deficiency is offset by the microcytic effect of iron deficiency. In such cases, a physician may rule out B12 deficiency, whereas patients may be deficient in both B12 and iron. Practically, while elevated MCV is a sure sign of B12 deficiency, due to the above-described nuances, MCV should be considered the least reliable marker of B12 status. Thus, normal MCV should never be used to rule out B12 deficiency. Normal MCV has been determined to be $87 \pm 7 \ \mu m^3$ [18].

Homocysteine

Hcy is a useful marker of B12 status. B12, along with several other B-vitamins, is essential in Hcy metabolism via methylene tetrahydrofolate reductase and methionine synthase enzymes. Hcy is derived from methionine. Thus, methionine intake is a factor in Hcy status. Folate, B12 and vitamin B6 are considered the most important nutrients in Hcy metabolism. In the United States and a number of other countries, following studies that documented the effectiveness of folic acid in preventing neural tube defects, some food items (e.g. flour) have been fortified with folic acid. Folic acid fortification resulted in an increase in the overall folic acid intake, which resulted in an improved Red Blood Cell (RBC) folate concentration. The higher folate status in the post-fortification era has also been associated with a reduction in Hcy concentration. Studies have shown that in the post-fortification era, vitamin B12 is the predominant cause of HHcy. Thus, in individuals with adequate folate intake, B12 is the predominant factor in Hcy status [19]. Considering that HHcy has been associated with increased risk of several diabetes co-morbidities, utilizing Hcy in clinical practice may have important implications in reducing risk of health problems among patients.

Although Hcy concentration of ≥ 15 µmol/L is extensively used by many researches and laboratories as indicative of HHcy, this cutoff is inadequate since even lower Hcy concentrations have been associated with health conditions that constitute common diabetic co-morbidities. For example, Jacobsen stated that, risk for coronary artery disease is represented by a continuum of total Hcy concentration, with a substantial risk occurring between 10 and 15 µmol/L [20]. Consistently, HHcy was defined as a concentration of at least 11.4 µmol/L for male participants and at least 10.4 µmol/L for female participants in the Third National Health and Nutrition Examination Survey [21]. Thus, patients with Hcy ≥ 10 µmol/L may require B12 therapy.

Holotranscobalamin II

Holo TCII constitutes the fraction of serum B12 that, with the help of the intrinsic factor, has been absorbed into Enterocytes. HoloTCII is the carrier of B12 into cells [22]. Thus, holoTCII is affected by intake and absorption of B12. HoloTCII is considered an early marker of

Table 2: Selected symptoms of vitamin B12 deficiency and/or hyperhomocysteinemia.

Category	Symptoms
Neurologica	Deterioration of the myelin, cognitive decline (e.g. memory loss), confusion, speech impairment (slurring), difficulty walking, inability to feel the ground, tingling, paresthesia, difficulty concentrating, numbness in both legs, mood alteration/swings, muscle cramps, paralysis, electric shock sensations, jerking movements of abdominal muscles, anxiety, depression, clumsiness, visual impairment, gait, shooting pain in calves, difficulty falling asleep, restless leg syndrome, optic neuropathy, subacute degeneration of the spine
Psychiatric	Disorientation, hyperactivity, decreased need for sleep, reckless and agitated behavior, social withdrawal, decreased interest, apathy, suspiciousness, hearing voices, hallucinations, anhedonia
Oral	Glossitis, pain and burning sensation in tongue, burning mouth syndrome, gradually progressive hoarseness, difficulty eating, red stains on inside of cheeks and tongue/glossitis/beefy tongue with U-shape streaks, oral epithelial dysplasia, cheilosis
Dermatological	Hyperpigmentation (blackish discoloration of the skin on knuckles, darkening of hands, feet, and tongue), skin lesions on feet, neck and upper and lower limbs, vitiligo foci (white patches on skin)
Hematological	Pancytopenia (low count of all blood cell types), macrocytic anemia, hyperhomocysteinemia
Other/rare	Anorexia, exercise intolerance, urinary incontinence, persistent watery diarrhea, normal blood pressure in supine position and rapid blood pressure drop in standing up position

inadequate B12 status [23]. According to Herbert, all cells have receptors for holoTCII while only the liver has receptors for Haptocorrin [11]. Since B12 can only be delivered to cells via holoTCII, low holoTCII indicates functional B12 deficiency. Inadequate supply of B12 is the first step in developing B12 deficiency [24]. Low holoTCII may not always be accompanied by overt B12 deficiency symptoms. However, persistently inadequate holoTCII may eventually lead to the onset of B12 deficiency symptoms. Low holoTCII indicates an inadequate supply of B12 into cells. This may occur regardless of B12 stores in the liver. In fact, low holoTCII may occur even when liver B12 stores are not depleted and serum/plasma B12 is above normal. Thus, using holoTCII will help in detecting functional B12 deficiency regardless of serum B12 values. The majority of researchers used holoTCII < 35 pmol/L as indicative of inadequate B12 supply/functional B12 deficiency. However, higher values have also been proposed. For example, Herrmann and Obeid suggested that individuals with holoTCII between 23 pmol/L and 75 pmol/L should be checked for MMA concentration to confirm B12 status [25], while Lloyd-Wright, et al. suggested that B12 deficiency is unlikely when holoTCII is > 50 pmol/L [26].

Methylmalonic Acid

MMA is a sensitive and specific marker of B12 status. B12 is a cofactor in the methylmalonyl-CoA mutase, an enzyme that is essential in methionine metabolism [27]. This enzyme metabolizes methymalonyl Co-A to succinyl Co-A. Thus, B12 deficiency will result in elevated MMA concentration. MMA concentration can be assessed in serum/plasma or in urine. Serum/plasma MMA may be affected by renal failure, thyroid disease, and small bowel bacteria overgrowth. Further, it can also be affected by an inborn error of metabolism that affects methylmalonate CoA. Thus, although it is possible to assess MMA concentration in serum/plasma, urinary MMA (uMMA) is considered more reliable and less invasive [28,29]. Since MMA is affected by kidney function, uMMA is adjusted for creatinine concentration (MMA/creatinine). Also, uMMA is affected by food intake. Thus, in order to obtain the most reliable results, fasting urine samples should be evaluated [30]. A value of serum/plasma MMA > 271 nmol/L or > 260 nmol/L is considered indicative of elevated MMA. Normal uMMA is < 4.0 μmol/L. More than one cutoffs for normal uMMA/creatinine has been proposed (e.g. < 4.8 mmol/mol creatinine, < 3.2 mmol/mol creatinine, 2.0 mmol/mol creatinine and 1.5 mmol/mol creatinine) [27,30,31]. Research findings showed that urinary MMA > 3.5 mmol/mol creatinine correlates with diabetic polyneuropathy [28].

Assessment of B12 status among patients with pernicious anemia

Pernicious anemia is a condition in which the gastric parietal cells fail to synthesis the intrinsic factor, which is essential for B12 absorption. A positive test for intrinsic factor antibodies may be a useful assessment method in some patients with pernicious anemia [32]. However, some individuals do test positive for the intrinsic factor antibody without actually having pernicious anemia. Parietal cell antibody test constitutes another, more accurate, assessment method. It is believed that up to 90 percent of patients with pernicious anemia will have a positive parietal cell antibody test [33]. Both the intrinsic factor antibody and the parietal cell antibody may detect the presence of antibodies in the stomach secretions and in serum.

Other useful assessment considerations

Vitamin B12 deficiency causes pancytopenia [34]. Thus, low normal or below normal platelet, and/or white blood cell count may be indicative of B12 deficiency. Such manifestations are often seen even among patients with "normal" values of vitamin B12 (often around or slightly above 200 pg/ml). Hypersegmentation of neutrophils may also be present among patients with B12 deficiency. However, the same may be true in case of folate or iron deficiency. Patients with B12 deficiency also can have low hemoglobin and hematocrit values, while in the same time having relatively high ferritin. Such patients may mistakenly be diagnosed with iron deficiency, while in reality, low hemoglobin and, in the same time, high ferritin are hallmarks of B12 deficiency. This biochemical findings is a result of inability to synthesize hemoglobin due to B12 deficiency, a phenomenon that can be called iron-trap. Since, as described above, some of the biomarkers of B12 status mentioned above are affected by factors other than B12 status it would be prudent, in clinical practice, to follow advice suggested by Herbert (as well as others) to utilize more than one assessment methods in order to obtain reliable B12 status [11,35]. Considering that HHcy is associated with several diabetes co-morbidities and that uMMA may be considered the most reliable diagnostic tool to assess B12 status, ideally, clinicians should obtain Hcy along with uMMA in order to obtain the best picture of B12 status. On the other hand, using serum/plasma B12 and MCV, due to the limitations described above, may not give a reliable picture of B12 status. Thus, practically, with the exception of serum/plasma B12 and MCV, any combination of two of the above described assessment methods (e.g. serum B12 and Hcy or serum B12 and uMMA) should give more reliable picture of B12 status than any single assessment method.

B12 replacement therapy

Treatment of B12 deficiency consists of either intramuscular injections or the use of B12 supplements. Nasal B12 sprays and toothpaste fortified with B12 are also available but not nearly as commonly used to treat

B12 deficiency. The most frequently applied intramuscular injection dose is 1,000 µg [36]. Lower doses (e.g. 100 µg or 250 µg) are sometimes used to treat children. There is wide variation in the frequency of intramuscular injections, from daily to weekly in the onset of treatment and monthly as follow up treatment. Daily or even every other day injections are seldom used for more than the first week of treatment. Monthly injections are used to maintain adequate B12 status. A supplemental B12 dose of 1000 µg/day is just as effective as therapy with intramuscular injection [36]. However, when supplements are utilized, patients would need to use them daily for a longer period of time than when injections are utilized. Supplemental B12 therapy has several advantages and one disadvantage. They include reduced cost of treatment and somewhat consistent supply of the vitamin. Anecdotal evidence shows that the benefits of monthly B12 injections wear off. This results in patients experiencing considerable improvements in the way they feel in the period of a few days after the injection and considerable regression of such feeling toward the next scheduled injection. In addition, ingested B12 tablet can be done with no supervision of a medical professional. The disadvantage has to do with the lack of standards and lack of control over the quality of supplements. More than one forms of B12 are available in intramuscular preparations and as oral supplements. They include at least cyanocobalamin, methylcobalamin, and hydroxocobalamin. All of the above forms of B12 have been shown to be effective in treating B12 deficiency. Kancherla, et al. found that patients receiving metformin therapy who also used oral multivitamin supplements had a 50% higher serum B12 or about 161 pmol/L higher serum concentration, compared to those patients who did not use multivitamin supplements [37]. Only 4% of those taking multivitamin supplements had subnormal B12 concentration compared with 15% among non-multivitamin supplements users. On the other hand, Reinstatler, et al. concluded that 6 µg of B12 found in most multivitamin supplements is insufficient for diabetic patients taking metformin [38]. Diabetic patients who ingested less than 6 µg per day of vitamin B12 from supplements had nearly 8 times higher risk of deficiency of this vitamin compared to those who ingested a dose greater than 25 µg/day or higher. Thus, a long-term use of oral B12 supplements in a dose of 25 µg/day might be needed to maintain adequate B12 status among these individuals. Patients who use other medications, such as aspirin or those that affect gastric acidity may need to utilize supplements with higher doses (e.g. 100 µg or 250 µg) [39]. The same may be true of elderly patients with diabetes.

Conclusions

Both metformin dose and duration of treatment are associated with reduced B12 status. In the NHANES data 5.8% of metformin users had B12 < 148 pmol/L with an additional 16.2% having a "borderline" deficiency (B12 between 148 and 221 pmol/L). Thus, at least 22% of metformin users in that study had inadequate B12 status. Symptoms of inadequate B12 may occur among individuals with even higher serum B12. As indicated in Table 1 serum B12 < 300 pmol/L may already indicate an inadequate B12 status. In 2010, about 1.9 million individuals 20 years and older were newly diagnosed with diabetes. Metformin is prescribed to approximately 65% of newly diagnosed patients [40]. Thus, considering that over 29 million individuals in the United States have diabetes and about 86 million have pre diabetes it is likely that an inadequate status resulting from the use of metformin is a problem among several millions of individuals [41].

The mechanism of how metformin-induced malabsorption of B12 is not 100% clear. More than one mechanisms have been proposed. According to Bell, the current and more likely explanation has to do with the fact that metformin effects the calcium-dependent membrane action in the terminal ileum, a mechanism on which B12 depends for absorption [42]. This hypothesis is supported by the fact that taking calcium supplements may reverse the malabsorptive effect of metformin.

Considering the effect of metformin treatment on B12 status and the fact that many diabetes patients are at a high risk of B12 deficiency due to other reasons, such as the use of other medications or age, and considering the impact of HHcy on diabetic co-morbidities, and the fact that B12 status is the main determinant of Hcy concentration for many individuals, it is prudent of the American Diabetes Association to recommend a routine check for B12 status. Routine screening, when appropriate B12 status analyses are performed and interpreted, would enable physicians to identify individuals with low B12 at early stage. Treatment with B12 injections or supplements would help in reducing risk of low B12 and HHcy-related symptoms. Screening older adults, elderly and those taking multiple medications at the time of diabetes diagnosis and/or those with impaired fasting glucose and/or impaired glucose tolerance would identify patients who may have low B12 prior to the unset of metformin therapy. They may need to be treated with B12 concomitant with metformin. Such practice would reduce pain and suffering of diabetic patients. It would also decrease medical cost associated with treatments of diabetic co-morbidities.

Acknowledgement

References

1. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, et al. (2006) Management of hyperglycemia in type 2

diabetes: A consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 29: 1963-1972.

2. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, et al. (2007) American Diabetes Association. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes Care 30: 753-759.

3. Niafar M, Hai F, Porhomayon J, Nader ND (2015) The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med 10: 93-102.

4. Liu Q, Li S, Quan H, Li J (2014) Vitamin B12 status in metformin treated patients: systematic review. PLoS One 9: e100379.

5. American Diabetes Association (2017) Standards of medical care in diabetes. Diabetes Care 40.

6. Xu C, Wu Y, Liu G, Liu X, Wang F, et al. (2014) Relationship between homocysteine level and diabetic retinopathy: a systematic review and meta-analysis. Diagn Pathol 9: 167.

7. Li J, Zhang H, Shi M, Yan L, Xie M (2014) Homocysteine is linked to macular edema in type 2 diabetes. Curr Eye Res 39: 730-735.

8. Wickramasinghe SN, Fida S (1993) Correlations between holo-transcobalamin II, holo-haptocorrin, and total B12 in serum samples from healthy subjects and patients. J Clin Pathol 46: 537-539.

9. Obeid R, Shannan B, Herrmann W (2011) Advanced glycation end products overload might explain intracellular cobalamin deficiency in renal dysfunction, diabetes and aging. Med Hypotheses 77: 884-888.

10. Langan RC, Zawistowski KJ (2011) Update on Vitamin B12 Deficiency. Am Fam Physician 83: 1425-1430.

11. Herbert V (l988) Vitamin B-12: plant sources, requirements, and assay. Am J Clin Nutr 48: 852-858.

12. Gröber U, Kisters K, Schmidt J (2013) Neuroenhancement with vitamin B12-underestimated neurological significance. Nutrients 5: 5031-5045.

13. Schwarz J, Morstadt E, Dura A, Wintgens KF, Hartmann K, et al. (2015) Biochemical Identification of Vitamin B12 Deficiency in a Medical Office. Clin Lab 61: 687-692.

14. Vogiatzoglou A, Oulhaj A, Smith AD, Nurk E, Drevon CA, et al. (2009) Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. Clin Chem 55: 2198-2206.

15. Salomon LR (2011) Diabetes as a Cause of Clinically Significant Functional Cobalamin Deficiency. Diabetes Care 34: 1077-1080.

16. Smith AD, Refsum H (2012) Do we need to reconsider blood level of vitamin B12? J Intern Med 271: 179-182.

17. Pawlak R, Parrott SJ, Raj S, Cullum-Dugan D, Lucus D (2012) Understanding vitamin B12. American Journal of Lifestyle Medicine 7.

18. Sarma RP (1990) Red cell indices. In: Walker HK, Hall WD, Hurst JW, Clinical Methods: The History, Physical, and Laboratory Examinations. (3rd edn), Butterworths, Boston, USA.

19. Green R, Miller JW (2005) Vitamin B12 deficiency is the dominant nutritional cause of hyperhomocysteinemia in a folic acid-fortified population. Clin Chem Lab Med 43: 1048-1051.

20. Jacobsen DW (1998) Homocysteine and vitamins in cardiovascular disease. Clin Chem 44: 1833-1843.

21. Selhub J, Jacques PF, Rosenberg IH, Rogers G, Bowman BA, et al. (1999) Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991-1994): population reference ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med 131: 331-339.

22. Quadros EV, Regec AL, Khan FK, Quadros L, Rpthenberg SP (2003) Transcobalamin II synthesized in the intestinal villi facilitates transfer of cobalamin to the portal blood. Physiol Rev 83: 871-932.

23. Herbert V, Fong W, Gulle V, Stopler T (1990) Low holotranscobalamin II is the earliest serum marker for subnormal vitamin B12 (cobalamin) absorption in patients with AIDS. Am J Hematol 34: 132-139.

24. Herbert V (1994) Staging vitamin B-12 (cobalamin) status in vegetarians. Am J Clin Nutr 59: 1213-1222.

25. Herrmann W, Obeid R (2013) Utility and limitations of biochemical markers of vitamin B12 deficiency. Eur J Clin Invest 43: 231-237.

26. Lloyd-Wright Z, Hvas AM, Møller J, Sanders TA, Nexø E (2003) Holotranscobalamin as an indicator of dietary vitamin B12 deficiency. Clin Chem 49: 2076-2078.

27. Bjørke Monsen AL, Ueland PM (2003) Homocysteine and methylmalonic acid in diagnosis and risk assessment from infancy to adolescence. Am J Clin Nutr 78: 7-21.

28. Sun AL, Ni YH, Li XB, Zhuang XH, Liu YT, et al. (2014) Urinary methylmalonic acid as an indicator of early vitamin B12 deficiency and its role in polyneuropathy in type 2 diabetes. J Diabetes Res 2014: 921616.

29. Norman EJ (1999) Urinary methylmalonic acid/creatinine ratio: a gold standard test for tissue vitamin B12 deficiency. J Am Geriatr Soc 47: 1158-1159.

30. Kwok T, Cheng G, Lai WK, Poon P, Woo J, et al. (2004) Use of fasting urinary methylmalonic acid to screen for metabolic vitamin B12 deficiency in older persons. Nutrition 20: 764-768.

31. Matchar DB, Feussner JR, Millington DS, Wilkinson RH Jr, Watson DJ, et al. (1987) Isotope-dilution assay for urinary methylmalonic acid in the diagnosis of vitamin B12 deficiency; a prospective clinical evaluation. Ann Intern Med 106: 707-710.

32. Khan S, Del-Duca C, Fenton E, Holding S, Hirst J (2009) Limited value of testing for intrinsic factor antibodies with negative gastric parietal cell antibodies in pernicious anaemia. J Clin Pathol 62: 439-441.

33. Bizzaro N, Antico A (2014) Diagnosis and classification of pernicious anemia. Autoimmun Rev 13: 565-568.

34. Halfdanarson TR, Walker JA, Litzow MR, Hanson CA (2008) Severe vitamin B12 deficiency resulting in pancytopenia, splenomegaly and leukoerythroblastosis. Eur J Haematol 80: 448-451.

35. Hannibal L, Lysne V, Bjørke-Monsen AL, Behringer S, Grünert SC (2016) Biomarkers and Algorithms for the Diagnosis of Vitamin B12 Deficiency. Front Mol Biosci 3: 27.

36. Stabler SP (2013) Vitamin B12 deficiency. NEJM 368: 149-160.

37. Kancherla V, Garn JV, Zakai NA, Williamson RS, Cashion WT (2016) Multivitamin Use and Serum Vitamin B12 Concentrations in Older-Adult Metformin Users in REGARDS, 2003-2007. PLoS One 11: e0160802.

38. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP Jr (2012) Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements. Diabetes Care 35: 327-333.

39. Van Oijen MG, Laheij RJ, Peters WH, Jansen JB, Verheugt FW (2004) BACH study. Association of aspirin use with vitamin B12 deficiency (results of the BACH study). Am J Cardiol 94: 975-977.

40. https://www.diabetesselfmanagement.com/blog/isnt-metformin-prescribed/

41. Centers of Disease Control and Prevention (2014) National Diabetes Statistics Report.

42. Bell DS (2010) Metformin-induced Vitamin B12 Deficiency Presenting as a Peripheral Neuropathy. South Med J 103: 265-267.

Pawlak. J Fam Med Dis Prev 2017, 3:05T

• Page 7 of 7 •