ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 8, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

PREVALENCE AND RISK FACTORS OF VITAMIN B_{12} DEFICIENCY AMONG PATIENTS WITH TYPE II DIABETES ON METFORMIN: A STUDY FROM NORTHERN REGION OF UNITED ARAB EMIRATES

MOAYAD SHAHWAN[1]*, NAGEEB HASSAN[1], ADEL NOSHI[1], NAHEED BANU[2]

[1]Department of Clinical Sciences, College of Pharmacy & Health Sciences, Ajman University, Ajman, UAE. [2]Department of Physical Therapy, College of Medical Rehabilitation, Qassim University, Buraydah, Qassim, Saudi Arabia. Email: moyad76@hotmail.com

Received: 19 March 2018, Revised and Accepted: 28 April 2018

ABSTRACT

Objectives: This study focused on the prevalence of Vitamin B_{12} deficiency in UAE patients with type 2 diabetes mellitus (T2DM) who were treated with or without metformin.

Methods: A cross-sectional study was conducted on 213 patients having diabetes type II were randomly selected to be part of the study in Northern Regions of the UAE, from June 2014 to February 2015. The patients aged >45 years and who had taken metformin for at least 3 months were recruited with regular follow-up at the outpatient clinic. The patients were included in a survey after which they had their serum B_{12} levels measured. Serum B_{12} levels <150 pg/ml are defined as the B_{12} deficiency.

Results: About 48% of diabetic patients had confirmed the B_{12} insufficiency through laboratory tests. The patients on metformin had statistically lower values of B_{12} (p=0.002). The majority of participants n (%) took metformin had neuropathy, hypertension, dyslipidemia, numbness or paresthesia, and depression, or mood changes 140 (70%), 183 (91.5%), 134 (67%), 136 (68%), 161 (80.5%), and 120 (60%), respectively.

Conclusion: Our study shows that for the patients with T2DM, long-term treatment with metformin is associated with higher chances of developing Vitamin B_{12} deficiency. Clinicians should, therefore, recognize this significant element and should screen diabetics who are on metformin treatment for any B_{12} insufficiency, which may be hidden, especially patients coming with neurologic symptoms.

Keywords: Type 2 diabetes, Metformin, B_{12} deficiency.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i8.26052

INTRODUCTION

The UAE is ranked 16th worldwide, with 19% of the UAE population living with diabetes [1]. These statistics indicate that the region has high-risk factors for diabetes, mostly related to rising obesity rates and physical inactivity. A sedentary lifestyle and bad eating habits are cited as the main causes of the increasing prevalence of type 2 diabetes mellitus (T2DM) in the UAE. It is becoming increasingly clear that T2DM is associated with decreasing level of activity and an increasing prevalence of obesity.

According to the World Health Organization, the number of T2DM patients is expected to double within the next 25 years.

Metformin's best known and most feared side effect, i.e., lactic acidosis almost never occurs if metformin is used appropriately [2]. The common side effects of metformin are gastrointestinal such as abdominal distress, soft stools, and diarrhea. In general, these side effects appear shortly after the initiation of metformin and promptly disappear after discontinuation. However, insidious or asymptomatic side effects resulting from long-term treatment, such as Vitamin B_{12} deficiency, may not be easily detected without close attention. It is important to recognize the clinical consequences of Vitamin B_{12} deficiency. Vitamin B_{12} which is a water-soluble vitamin plays a very fundamental role in DNA synthesis, optimal hematopoiesis, and neurological function. The clinical picture of Vitamin B_{12} deficiency, hence, is predominantly of features of hematological and neurocognitive dysfunction [3].

Several studies have screened outpatients taking metformin for Vitamin B_{12} deficiency. The association between metformin treatment and impaired Vitamin B_{12} status dates back to the 1970s when the interaction was first proposed [4], and estimates of prevalence were stated to be close to 30%. Since then, the prevalence of metformin-associated Vitamin B_{12} deficiency has been reported in many different studies; however, the ranges of incidence in such populations vary by a large amount. While many studies estimate the rate of prevalence to be around 10–20% of treated patients [5,6], other studies have reported level as low as 5% of treated patients [7] and level higher than 30% [8].

The mechanism by which metformin therapy causes Vitamin B_{12} deficiency is not clear, but it is thought to be due to either alteration in small bowel motility, which stimulate small bowel bacterial overgrowth and subsequent Vitamin B_{12} deficiency, or by directly decreasing Vitamin B_{12} absorption [9,10]. The absorption of the Vitamin B_{12} intrinsic factor complex by cells of the terminal ileum is calcium dependent, and metformin alters intracellular handling of calcium, thereby reducing absorption [9,10]. The later theory is supported by the fact that the administration of calcium reverses metformin-induced Vitamin B_{12} deficiency [10].

Although the clinical significance of Vitamin B_{12} deficiency related to metformin treatment is debatable, monitoring for Vitamin B_{12} has been recommended for patients with T2DM, especially those on long-term metformin treatment [11]. The extended use of metformin, accompanied by Vitamin B_{12} deficiency, may lead to increasing the considerable problem of peripheral neuropathy in non-insulin-dependent DM patients. Neuropathy, being an impending health abnormality occurring due to Vitamin B_{12} deficiency, affects around 30% diabetics who are over 40 years of age and states about having a diminished sensory perception in their feet [12]. A proposed

mechanism for these neurological effects is the disturbance of myelin synthesis due to impaired production of methionine [13]. Therefore, it is worthwhile to consider the prevalence of Vitamin B_{12} deficiency among the growing T2DM population.

As metformin has been prescribed worldwide and treatment periods increase, the prevalence of metformin-induced Vitamin B_{12} deficiency may have also significantly increased. However, the relationship between metformin use and Vitamin B_{12} deficiency in the UAE population has not been widely investigated. This study focused on the prevalence of Vitamin B_{12} deficiency in UAE patients with T2DM who were treated with or without metformin.

METHODS

Study design, participants, and procedures

This study is a descriptive, cross-sectional study. A total of 213 participants were randomly selected to be part of the study in Northern Regions of the UAE, from June 2014 to February 2015. 200 patients who fulfilled the inclusion criteria were included in the study.

All patients aged 45 years or older who were being treated for T2DM at the outpatient clinic were eligible for inclusion, regardless of metformin use. We excluded all participants with diabetes after necrotic pancreatitis, late-onset autoimmune diabetes of adults, and pure vegetarians had a history of pernicious anemia, chronic renal insufficiency defined by a creatinine 3.0, prior bariatric surgery, gastrectomy, B_{12} supplementation with B_{12} shots or an oral Vitamin B_{12} dose of 500 mcg/day, prior ileum resection, or Crohn's disease.

Before and during their regular scheduled visit to their treating internist, patients were informed about this study. Subsequently, patients could consult one of the investigators to receive more information and sign informed consent when they had agreed to participate.

During this visit, a structured questionnaire was used to collect the data. It consisted of two parts; first part included demographic data and the second part included 17 study questions which consist of information about the level of Vitamin B_{12}, duration of diabetes, hemoglobin A1c, metformin use, and metformin duration. Data on additional patient characteristics and diabetes complications were retrospectively searched for in the patient records. Vitamin B_{12} level <150 pmol/L was considered deficient, whereas Vitamin B_{12} level ≥150 pmol/L was considered normal.

Statistical analysis

All statistics were done with SPSS software version 20.0 (SPSS, Inc., Chicago, IL, USA). Associations between categorical values and B_{12} deficiency were done with two analyses. Associations between continuous variables and Vitamin B_{12} deficiency were done with Student's t-test. Student's *t*-test was also used to determine associations between metformin use and serum level of B_{12}. A multivariate analysis using logistic regression was used to identify factors independently associated with Vitamin B_{12} deficiency. Covariates chosen for the multivariate model were known or hypothesized biologic factors that would affect Vitamin B_{12} deficiency. $p<0.05$ was considered statistically significant.

RESULTS

A total of 213 patients enrolled in the study. 13 patients were excluded: Nine were younger than 45 years of age and four were taking supplemental B_{12} 500 mcg/day. The remaining 200 patients were included in the final analysis.

Table 1 summarizes the participants demographic data. The mean (± standard deviation [SD]) age of the patients was 49.5 (±7.8) years; duration of diabetes 3.48 (±3.01) years; body mass index (BMI) was 28.51 (±5.25); hemoglobin A1c (HbA1c) was 6.7 (±0.62); metformin duration 3.35 (±1.88) years, and serum Vitamin B_{12} level 158.5 (±43.36) pmol/L and ranged from 62 to 351 pmol/L among all patients. There were 96 (48%) patients with Vitamin B_{12} deficiency (<150 pmol/L) and 104 (52%) patients with normal Vitamin B_{12} level (≥150 pmol/L).

Table 1: Characteristics of outpatient patients with type 2 diabetes

Characteristics (N=200)	Value
Age (years) (mean±SD)	49.5±7.8
Serum Vitamin B_{12} pmol/L (mean±SD)	158.54±43.36
Duration of diabetes (years) (mean±SD)	3.48±3.01
Body mass index (kg/m2) (mean±SD)	28.51±5.25
Hemoglobin A1c (%) (mean±SD)	6.73±0.62
Metformin duration (years) (mean±SD)	3.35±1.881
Metformin use n (%)	140 (70)
Gender	
Male n (%)	102 (51)
Female n (%)	98 (49)
Insulin use n (%)	43 (21.5)
Sulfonylurea use n (%)	76 (37)
Retinopathy n (%)	40 (20)
Neuropathy n (%)	183 (91.5)
Nephropathy n (%)	31 (15.5)
Hypertension n (%)	134 (67)
Ischemic heart disease n (%)	14 (7)
Dyslipidemia n (%)	136 (68)
Numbness or paresthesia n (%)	161 (80.5)
Depression or mood changes n (%)	120 (60)
Memory changes n (%)	43 (21.5)

SD: Standard deviation

The majority of participants n (%) took metformin had neuropathy, hypertension, dyslipidemia, numbness or paresthesia, and depression, or mood changes 140 (70%), 183 (91.5%), 134 (67%), 136 (68%), 161 (80.5%), and 120 (60%), respectively. Regarding gender more than half of the patients were male 102 (51%) and 98 (49%) were female.

Table 2 summarizes key variable comparisons between patients with and without B_{12} deficiency.

Regarding continuous variables, age, diabetes duration, metformin duration, BMI, and HbA1c were not associated with B_{12} deficiency ($p>0.05$).

When a second X^2 was performed defining at risk for Vitamin B_{12} deficiency as any serum level <150 pmol/L, the results indicated that patients using metformin were statistically at higher risk for Vitamin B_{12} deficiency (odds ratio, 2.64; 95% confidence interval, 1.40–4.99). A series of further analysis were subsequently performed to determine whether metformin duration was associated with B_{12} deficiency. In this study population, there was no statistically significant association between the average prescribed metformin duration for patients with B_{12} deficiency (mean±SD) 3.29±1.93 and patients without B_{12} deficiency 3.43±1.84 ($p=0.66$).

A *t*-test was conducted to determine whether any difference existed in the B_{12} level of individuals using metformin versus those who were not. Patients taking metformin had statistically significant lower level of B_{12} (123.71 pmol/L vs. 190.69 pmol/L; $p<0.001$) (Table 3).

The prediction equation

Vitamin B_{12} Deficiency= 1.336 + 0.003 (BMI) − 0.001 (Age) + 0.003 (DM duration) + 0.006 (Metformin duration)

BMI, age, duration of diabetes, and metformin duration values (independent variables) were included in multiple regression test as predictors for Vitamin B_{12} deficiency level (outcome dependent variable). Only metformin duration was the significant predictor ($p<0.0001$). We randomly selected 10 patients from our list to calculate the predicated Vitamin B_{12} deficiency. The results were very predicative as follows:

Table 2: Bivariate associations with Vitamin B_{12} deficiency

Continuous variables	Vitamin B_{12} deficiency Yes (n=96)	Vitamin B_{12} deficiency No (n=104)	p value
Age, years (Mean±SD)	49.97±7.76	49.04±7.80	0.39
Diabetes duration - years (Mean±SD)	3.63±3.113	3.34±2.915	0.49
Metformin duration (Mean±SD)	3.29±1.925	3.43±1.84	0.66
Body mass index (kg/m^2) (Mean±SD)	28.42±5.21	28.6±5.3	0.80
Hemoglobin A1c (%) (Mean±SD)	6.8±0.66	6.7±0.58	0.24
Categorical variables (%)			
Gender			
Male	(50)	(51.9)	0.786
Female	(50)	(48.1)	
Metformin use			
Yes	(80.2)	(60.6)	0.002
No	(19.8)	(39.4)	
Insulin use			
Yes	(14.6)	(27.9)	0.022
No	(85.4)	(72.1)	
Sulfonylurea			
Yes	(26)	(47.1)	0.002
No	(74)	(52.9)	
Retinopathy			
Yes	(22.9)	(17.3)	0.322
No	(77.1)	(82.7)	
Neuropathy			
Yes	(88.5)	(94.2)	0.144
No	(11.5)	(5.8)	
Nephropathy			
Yes	17.7)	(13.5)	0.407
No	82.3)	(86.5)	
Hypertension			
Yes	(62.5)	(71.2)	0.193
No	(37.5)	(28.8)	
Ischemic heart disease			
Yes	(7.3)	(6.7)	0.877
No	(92.7)	(93.3)	
Dyslipidemia			
Yes	(65.6)	(70.2)	0.489
No	(34.4)	(29.8)	
Numbness or paresthesia			
Yes	(76)	(84.6)	0.126
No	(24)	(15.4)	
Depression or mood changes			
Yes	(58.3)	(61.5)	0.644
No	(41.7)	(38.5)	
Memory changes			
Yes	(21.9)	(21.2)	0.901
No	(78.1)	(78.8)	

SD: Standard deviation

The mean percentage of the prediction was 81% that is high enough for prediction equation to be a powerful tool for reproducing testable results.

Multivariate logistic regression analysis showed that in this study sample, metformin duration significantly contributed ($p<0.0001$) to the predication of Vitamin B_{12} deficiency among patients with T2DM. Age and metformin use were insignificant predictors of Vitamin B_{12} deficiency (Table 4).

DISCUSSION

In this cross-sectional study, we are aware of that was specifically designed to define the prevalence of B_{12} deficiency in patients with T2DM. In our cohort, we identified 48% of T2DM patients with B_{12} deficiency [14]. In multivariate models, metformin use was a positive, and age use was a negative predictor of this deficiency. Of all patients (regardless of metformin use), 15.5% were nephropathy and 91.5% had neuropathy. It is possible that the chronic disease T2DM in itself is sufficient to explain the nephropathy in this population. Furthermore, 3.29 years of metformin use was related to a higher prevalence of neuropathy than when no metformin was used.

Our study showed that the prevalence of B_{12} deficiency among secondary care treated T2DM patients was 80% in metformin users (median metformin use 3.29 years) and 20% in non-metformin users. Previous studies assessing T2DM patients on metformin have reported the prevalence of Vitamin B_{12} deficiency to range from 5.6% to 33%. This wide variation in the reported prevalence could probably be explained by the varied study definitions of Vitamin B_{12} deficiency. Tomkin, in 1972, was the first described cohort in T2DM patients using metformin for <5 years, a prevalence of 5.6% among metformin users was found [15].In the cross-sectional study by Pflipsen *et al*. found a prevalence of 22.6% among patients with T2DM using metformin, in a primary care setting [16]. In a study by De Jager *et al*. found a 9.9% prevalence of B_{12} deficiency in patients treated with metformin for 4.3 years [11]. Reinstatler *et al*. defined B_{12} deficiency as B_{12} ≤148 pmol/l and found a prevalence of B_{12} deficiency of 5.8% in a cohort of patients followed for 6 years [7]. A recent cross-sectional study documented a high prevalence of Vitamin B_{12} deficiency of 33% among adult patients with T2DM by Qureshi *et al*. Vitamin B_{12} deficiency was defined as serum Vitamin B_{12} concentration <150 pg/mL [17]. It was observed that the prevalence of decreased serum Vitamin B_{12} status in metformin-treated

Table 3: Multiple regression analysis for possible predicting factors for Vitamin B12 deficiency

Variables	Unstandardized coefficients		Standardized coefficients	t	p value
	Slope	Std. error	Beta		
(Constant)	1.336	0.261		5.120	0.000
BMI	0.003	0.006	0.035	0.550	0.583
Age	−0.001	0.005	−0.020	−0.280	0.780
Duration of DM	0.003	0.012	0.020	0.287	0.774
Duration metformin treat years	0.006	0.001	0.457	7.089	0.000

DM: Diabetes mellitus, BMI: Body mass index

Table 4: Randomly selected 10 patients from our list to calculate the predicated Vitamin B_{12} deficiency

Patient serial number	Vitamin B_{12} deficiency level (actual)	Vitamin B_{12} deficiency level (predicted)	Actual/predicted (×100)
1	112	136	82
2	124	136	91
3	137	137	100
4	136	137	99
5	133	141	94
6	108	137	79
7	117	142	82
8	62	141	44
9	106	139	76
10	92	146	63

patients in our study was higher than those in the previous studies. This comparison must be interpreted with caution as there are other factors that may affect the serum Vitamin B_{12} of these patients which were not addressed in this study (diet, drug interactions, etc.). However, it is interesting to note that our sample population had a better Vitamin B_{12} status than that of a population without T2DM or metformin treatment.

Ting *et al*. studied risk factors of B_{12} deficiency in patients receiving metformin. The dose of metformin was the strongest independent predictor of Vitamin B_{12} deficiency, and a longer duration of treatment with metformin was associated with a higher prevalence [18]. This study shows an association between the decreasing B_{12} level and metformin duration. In accordance with the present study, while Reinstatler *et al*. found no clear increase in the prevalence of deficiency as the duration of metformin use increased [2].

The relatively high prevalence of B_{12} deficiency found in this study makes it likely that at least a portion of peripheral neuropathy cases in diabetic patients may be attributed to B_{12} deficiency. Previous studies have demonstrated that supplemental Vitamin B_{12} improved somatic and autonomic symptoms of diabetic neuropathy [19,20]. Testing for, and treating, B_{12} deficiency in those patients with neuropathy may lead to improved clinical outcomes. Clinical trials are needed to further evaluate this link.

The present study adds to this discussion by again defining a prevalence, confirms the influence of metformin on a Vitamin B_{12} deficiency, and shows that although metformin increases B_{12} deficiency rates, it does not increase odds for neuropathy after 3.29 years treatment with metformin. This finding argues against standard screening and/or supplementation of Vitamin B_{12} in metformin-treated T2DM patients. We would, therefore, like to plead for more research focusing on the consequences of a metformin-induced B_{12} deficiency to determine whether screening and supplementation are necessary.

Regarding the relation between the prevalence of B_{12} deficiency and the age, several studies demonstrated a prevalence of B_{12} deficiency in the elderly that ranges from 12% to 23% [21,22]. Although the prevalence of B_{12} deficiency in our diabetic patients was in line with these results, it is important to note that the average age of our population was approximately 10 years younger than the average age of the elderly volunteers enrolled in these other studies. In addition, both bivariate and multivariate analyses demonstrated that age was not significantly associated with B_{12} deficiency. This suggests that type 2 diabetes, not age, may account for the 48% prevalence of B_{12} deficiency.

Patients on chronic metformin therapy seem to be at increased risk for B_{12} deficiency. Its use is associated with lower serum Vitamin B_{12} level [14,24-26]. Several studies associate metformin use with established clinical B_{12} deficiency [17,26,27]. It has been reported that higher doses and longer treatment with metformin seem to be risk factors for such deficiency [28]. Although we found that patients using metformin had lower B_{12} level, we did not find metformin use to be associated with overt B_{12} deficiency. Our study was not designed nor powered to find these secondary associations. Current metformin use was associated with a significantly higher risk for B_{12} deficiency when defined as a serum B_{12} level <150 pmol/L. Patients with B_{12} level <150 pmol/L may be at risk for B_{12} deficiency because tissue deficiency may occur despite normal serum B_{12} level [22,29,30]. Identification of patients "at risk" for B_{12} deficiency as those with serum B_{12} <150 pmol/L may help the clinician define a level to test for B_{12} deficiency using specific tissue markers, especially among diabetics who are using metformin.

Our multivariate analysis looked for specific associations for B_{12} deficiency. It showed that only metformin duration seemed to be a significant protector in diabetic patients from B_{12} deficiency. Other factors known to increase risk for B_{12} deficiency, such as advanced age and duration of DM, were not significantly associated with B_{12} deficiency. Further, research needs to be conducted in large and well-designed studies on screening Vitamin B_{12} deficiency and also to look at the potential risk or protective factors for Vitamin B_{12} deficiency.

CONCLUSION

The prevalence of B_{12} deficiency in secondary care T2DM patients using metformin was estimated at 80%. The prevalence is significantly higher in patients treated with metformin compared with non-metformin users. Furthermore, metformin use does predict the deficiency of Vitamin B_{12} in diabetic patients' after 3.29 years treatment with metformin. Moreover, metformin use did lead to neuropathy.

AUTHOR'S CONTRIBUTION

Moayad Shahwan drafted and edited the manuscript, Nageeb Hassan interpreted the results, Adel Noshi did the data collection and statistical analysis, and Naheed Banu approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. International Diabetes Federation, IDF Diabetes Atlas. International Diabetes Federation, 6th. 2013. Available from: http://www.idf.org/diabetesatlas.
2. Dareen A, Abduelmula A, Moyad S, Monzer S. Evaluation of factors associated with inadequate glycemic control and some other health care indicators among patients with Type 2 diabetes in Ramallah, Palestine. Res J Pharm Biol Chem Sci 2013;4:445-51.
3. Oh R, Brown DL. Vitamin B_{12} deficiency. Am Fam Physician 2003;67:979-86.
4. Stowers JM, Smith OA. Vitamin-B_{12} and metformin. BMJ 1971;3:246-7.
5. Calvo Romero JM, Ramiro Lozano JM. Vitamin B(12) in Type 2 diabetic patients treated with metformin. Endocrinol Nutr 2012;59:487-90.
6. Centers for Disease Control and Prevention: National Diabetes Statistics Report: Estimates of Diabetes and its Burden in the United States; 2017. Available from: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf. [Last accessed on 2018 Jan 10]
7. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP Jr. Association of biochemical B_{12} deficiency with metformin therapy and vitamin B_{12} supplements: The national health and nutrition examination survey, 1999-2006. Diabetes Care 2012;35:327-33.
8. Nervo M, Lubini A, Raimundo FV, Faulhaber GA, Leite C, Fischer LM, *et al.* Vitamin B_{12} in metformin-treated diabetic patients: A cross-sectional study in Brazil. Rev Assoc Med Bras (1992) 2011;57:46-9.
9. Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: An old medication of new fashion: Evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. Eur J Endocrinol 2010;162:193-212.
10. Lineo M, Masia T, Binyane K, Ramathebane M. Assessment of patient counselling in diabetic and hypertensive patients in terms of patient knowledge about their medication, disease state and lifestyle modifications by pharmacy personnel at ldf clinic in Maseru. Int J Pharm Pharm Sci 2018;10:155-61.
11. de Jager J, Kooy A, Lehert P, Wulffelé MG, van der Kolk J, Bets D, *et al.* Long term treatment with metformin in patients with Type 2 diabetes and risk of vitamin B-12 deficiency: Randomised placebo controlled trial. BMJ 2010;340:c2181.
12. American Association for Clinical Chemistry. Vitamin B_{12} and Folate Deficiency. Available from: https://www.labtestsonline.org/conditions/vitamin-b12-and-folate-deficiencies. [Last updated on 2015; Last cited on 2017 10].
13. Jayesh BD, Snehal NM, Archana RJ. Diabetic nephropathy–genesis, prevention and treatment. Int J Pharm Pharm Sci 2014;S1:42-7.
14. Tomkin GH, Hadden DR, Weaver JA, Montgomery DA. Vitamin-B_{12} status of patients on long-term metformin therapy. Br Med J 1971;2:685-7.
15. Tomkin GH. Metformin and B_{12} malabsorption. Ann Intern Med 1972;76:668.
16. Pflipsen M, Oh R, Saguil A, Seehusen D, Topolski R. The prevalence of vitamin B_{12} deficiency in patients with Type 2 diabetes: A cross-sectional study. J Am Board Fam Med 2009;22:528-34.
17. Qureshi SA, Ainsworth A, Winocour PH. Metformin therapy and assessment for vitamin B_{12} deficiency: Is it necessary. Pract Diabetes 2011;28:302-4.
18. Ting R, Szeto C, Chan M, Ma K, Chow K. Risk Factors of vitamin B_{12} deficiency in patients receiving metformin. Arch Intern Med 206;166:1975-9.
19. Khan MA, Wakefield GS, Pugh DW. Vitamin B_{12} deficiency and diabetic neuropathy. Lancet 1969;2:768-70.
20. Yaqub B, Siddique A, Sulimani R. Effects of methylcobalamin on diabetic neuropathy. Clin Neurol Neurosurg 1992;94:105-11.
21. Pennypacker LC, Allen RH, Kelly JP, Matthews LM, Grigsby J, Kaye K, *et al.* High prevalence of cobalamin deficiency in elderly populations. J Am Geriatr Soc 1992;40:1197-204.
22. Lindenbaum J, Rosenberg IH, Wilson P, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr 1994;60:2-11.
23. Johnson MA, Hawthorne NA, Brackett WR. Hyperhomocysteinemia and vitamin B_{12} deficiency in elderly using Title IIIc nutrition services. Am J Clin Nutr 2003;77:211-20.
24. Filioussi K, Bonovas S, Katsaros T. Should we screen diabetic patients using biguanides for megaloblastic anemia. Aust Fam Phys 2003;32:383-4.
25. Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B_{12} and folic acid: A cross sectional study in patients with Type 2 diabetes mellitus. J Med Assoc Thai 2004;87:780-7.
26. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The multicenter metformin study group. N Engl J Med 1995;333:541-9.
27. American Diabetes Association Position Statement. Standards of medical care in diabetes. Diabetes Care 2012;35:S11-63.
28. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B_{12} deficiency in patients receiving metformin. Arch Intern Med 2006;166:1975-80.
29. Stabler SP. Screening the older population for cobalamin (Vitamin B_{12}) deficiency. J Am Geriatr Soc 1995;43:1290-7.
30. Hvas AM, Nexo E. Diagnosis and treatment of vitamin B_{12} deficiency—an update. Hematological 2006;91:1506-151.