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RESEARCH ARTICLE

Prevalence of Vitamin B12 Deficiency Among Chinese Patients with Type 2 Diabetes Mellitus Treated with Metformin in Primary Care in Hong Kong

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Abstract

Background: The aim was to evaluate the prevalence of vitamin B12 deficiency among Chinese patients with type 2 Diabetes Mellitus (DM) treated with metformin in primary care in Hong Kong. The associated factors of vitamin B12 deficiency were also analyzed.

Methods: This was a cross-sectional study. A randomized list of patients with type 2 DM treated with metformin who attended a general out-patient clinic from 1st December 2019 to 29th February 2020 was generated. Both serum total and active vitamin B12 levels would be checked and either one below the cut-off values would be defined as vitamin B12 deficiency.

Results: A total of 406 patients were recruited. The prevalence of metformin induced vitamin B12 deficiency was 22.4%. Higher metformin dosage was found to be a statistically significant associated factor for developing vitamin B12 deficiency. Compared with metformin dosage of less than 1000mg/day, the OR of developing vitamin B12 deficiency for 1500mg to less than 2000mg/day was 3.71 (95% C.I.: 1.10 to 12.54; p=0.04); and for 2000mg/day or above was 8.03 (95% C.I.: 2.76 to 23.34; p<0.01). Among the patients with metformin induced vitamin B12 deficiency, 23.1% of patients had anemia and only 9.9% had macrocytosis. Longer duration of diabetes, longer duration of use of metformin, diabetic control and the use of proton pump inhibitor (PPI) were not associated with vitamin B12 deficiency.

Conclusion: Patients taking high dose of metformin (≥1500mg/day) should be screened for vitamin B12 deficiency periodically in primary care even if they had no anaemia or macrocytosis.

Keywords: Type 2 Diabetes, Metformin, Vitamin B12 Deficiency, Primary Care

INTRODUCTION

According to the American Diabetes Association (ADA) guideline in 2019 [1] and the European Association for the Study of Diabetes (EASD) guideline in 2018 [2], metformin is the recommended monotherapy for patients with newly diagnosed type 2 DM unless there are contraindications. However, use of metformin is associated with potential adverse effects of vitamin B12 deficiency. Dated back to 1971, Tomkin et al already reported that vitamin B12 malabsorption was found in 30% of diabetic patients taking long-term metformin.[3] To date, a meta-analysis done by Chapman et al demonstrated an association between metformin usage and lower levels of vitamin B12 by 57pmol/L after just 6 weeks to 3 months of use, which could lead to frank deficiency or borderline status in type 2 DM patients.[4] Furthermore, the Diabetes Prevention Program Outcomes Study (DPPOS), one of the largest and longest studies of metformin treatment, reported a 13% increased risk of vitamin B12 deficiency per year of total metformin use.[5] Severe vitamin B12 deficiency can result in macrocytic anaemia, peripheral neuropathy, subacute combined degeneration of spinal cord, and mental-psychiatric changes.[6-8] As a result, the ADA guideline recommended periodic testing of vitamin B12 levels in metformin-treated patients.[1]

Early theory suggested the inhibitory effect of metformin on intestinal motility leading to small intestinal bacterial overgrowth, which in return affected the vitamin B12 absorption.[9] More recent theory suggested the antagonistic effect of metformin on the calcium-dependent ileal membrane

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action, thus affecting the B12-intrinsic factor complex uptake by the ileal cell surface receptors.[10] Previous studies have also suggested the combination of metformin and proton pump inhibitor (PPI) in association with vitamin B12 deficiency. [11, 12] Damião CP et al postulated that since gastric acid is required for vitamin B12 absorption, and the use of PPI would decrease the acid secretion by the parietal cells, thus increasing the chance of vitamin B12 deficiency.[12] However, results had been conflicting with other studies which failed to support this association. [13, 14]

Other risk factors for vitamin B12 deficiency include gastric diseases, pernicious anemia; decreased ileal absorption due to Crohn's disease, ileal resection; genetic causes like transcobalamin II deficiency; inadequate intake due to dietary insufficiency, vegetarian, alcohol abuse, patients older than 75 years [15], low socioeconomic level [16]; as well as chronic smoking [17], and obesity [18].

In Hong Kong, an observational, cross-sectional study done in geriatric diabetic patients found a higher prevalence of definite deficiency (29% vs. 5%) and possible deficiency (52% vs. 27%) in patients taking metformin. Definite deficiency was defined as serum vitamin B12 level of <150 pmol/ L, whereas possible deficiency as <220 pmol/L.[19] Another recent retrospective study reported that the prevalence of vitamin B12 deficiency in institutionalized elderly diabetic patients taking metformin was 53.2% compared with 31.0% of elderly diabetic patients not taking metformin and 33.3% of those without diabetes. [20] Looking at risk factors for metformin associated vitamin B12 deficiency, a nested case-control study found a significant association of vitamin B12 deficiency with dose and duration of metformin use. Each 1g per day metformin dose increment conferred an odds ratio (OR) of 2.88 (95% C.I.: 2.15-3.87) for developing vitamin B12 deficiency. Among those using metformin for 3 years or more, the adjusted OR was 2.39 (95% C.I.: 1.46-3.91) compared with those receiving metformin for less than 3 years.[14]

Direct tests of vitamin B12 status include serum total vitamin B12 and more recently, active vitamin B12 (also called holotranscobalamin). Active vitamin B12 is the fraction of vitamin B12 bound to transcobalamin that can be taken up from the blood into the cells of the body, so it represents the biologically active form of this vitamin. Currently there is no 'gold standard' test to define vitamin B12 deficiency. [7] Diagnostic test accuracy studies have reported greater diagnostic accuracy with using the active vitamin B12 assay compared with assays measuring other markers of vitamin B12 deficiencies. [21-26] Accordingly, The National Institute for Health and Care Excellence (NICE) published a report in 2015 commenting the active vitamin B12 assay should replace the current standard serum total vitamin B12 levels, particularly in situations of indeterminate or borderline serum total vitamin B12 results.[27]

Damião CP et al showed that among 52 metformin induced vitamin B12 deficient patients, anemia was seen in 12 (23.1%) and macrocytosis was seen in 5 (9.6%) patients only. The prevalence of anemia and mean corpuscular volume (MCV) level did not differ between patients with and without vitamin B12 deficiency, though macrocytosis was significantly more frequent in the vitamin B12 deficient group.[12] Another study by Ko SH et al revealed that metformin induced vitamin B12 deficient patients had higher rates of anemia than those without deficiency (27.6% versus 15%). However, there were no differences in the mean MCV between the two groups, and no vitamin B12 deficiencies were detected in those with MCV > 100 fL, which was used to define megaloblastic anemia.[13] Therefore, complete blood count cannot be used as a screening test for metformin related vitamin B12 deficiency.

In Hong Kong, most diabetic patients had follow-ups in the public sector and about sixty percent were under primary care. Most of the diabetic patients under primary care were taking metformin but there were no relevant studies on the prevalence of metformin induced vitamin B12 deficiency in primary care. There was also no aligned practice of periodic screening of vitamin B12 deficiency. This study was thus conducted with the aim to find out the prevalence and associated risk factors of vitamin B12 deficiency among Chinese diabetic patients treated with metformin in primary care, so as to provide evidence-based recommendations for the need of regular screening of vitamin B12 deficiency in this group of patients.

METHODOLOGY

This was a cross-sectional study. It was carried out in a Hospital Authority (HA) General Out-Patient Clinic. There were around 5,500 diabetic patients with regular follow-ups in the clinic. A list of all the patients who fulfilled the inclusion criteria from 1st December 2019 to 29th February 2020 was generated. Patients were randomly selected and invited to participate in the study with written consent signed during daily consultation in the five consultation rooms of the clinic until the required sample size was achieved. The inclusion and exclusion criteria were summarized as below:

Inclusion criteria:

1. Subjects with diagnosis of type 2 DM treated with metformin and attended the participating clinic regularly for follow up of diabetes

Exclusion criteria:

1. Patients with type 1 DM
2. Non-Chinese patients
3. Patients age less than 18 years old
4. Patients with pernicious anemia
5. Patients with Crohn's disease

6. Patients with history of gastrectomy or small bowel resection
7. Patients who have received oral or intramuscular vitamin B12 supplementation in the previous 3 months
8. Patients who cannot give consent or refuse to participate in the study

For those who fulfilled the inclusion criteria, a maximum of 10ml of bloods were taken to check for the serum total vitamin B12, active vitamin B12, blood haemoglobin (Hb) and MCV. Both the serum total and active vitamin B12 assays were performed by the Access® 2 immunoassay system and the UniCel® DxI 800 immunoassay system from Beckman Coulter respectively in the Haematology laboratory of a HA Hospital. The reference range of serum total vitamin B12 established by the laboratory was 133-675 pmol/L, while the reference range for active vitamin B12 was 26.7-176.1 pmol/L according to the reagent insert. Thus, serum total vitamin B12 level below 133 pmol/L or active vitamin B12 level below 26.7 pmol/L would be considered as vitamin B12 deficiency. Moreover, anemia was defined as Hb <13.5 g/dl for males and <11.5 g/dl for females; while macrocytosis was defined as MCV > 96 fL referenced from the same HA hospital.

There was no 'gold standard' test to define vitamin B12 deficiency as mentioned above.[7] Though active vitamin B12 was reported to have greater diagnostic accuracy [21-26], it was not widely available and many of the published studies were still using total vitamin B12 level to diagnose vitamin B12 deficiency. In our study, both serum total and active vitamin B12 levels would be checked and vitamin B12 deficiency would be defined as either total or active vitamin B12 levels below the cut-off values.

For patients found to have vitamin B12 deficiency, they would be called back for further evaluation and management which would include: assessment of symptoms and signs of vitamin B12 deficiency; further blood tests including anti-intrinsic factor (IF) and anti-parietal cell (PC) antibodies; referral to the internal medicine specialist out-patient clinic for further assessment of possible underlying gastric causes; and vitamin B12 replacement would be commenced. If patients refused for further blood tests to check for anti-IF and anti-PC antibodies, they would be counted as metformin induced vitamin B12 deficiency as we assumed vitamin B12 deficiency induced by metformin was more common than pernicious anemia and hence more likely in this group of patients.

Patients' data were collected from the computerized medical records which included: demographic data namely age and gender, body mass index (BMI), current smoking and drinking status, and socioeconomic status i.e. whether the patients were receiving Comprehensive Social Security Assistance (CSSA). The duration of DM, latest metformin daily dose, duration of metformin therapy, and concomitant use of PPI therapy at the time of blood collection were also collected. The dietary history namely vegetarian or non-vegetarian diet would be asked during the consultation.

Metformin induced vitamin B12 deficiency was defined after excluding possible dietary cause of vitamin B12 deficiency i.e. vegetarian, and those patients with positive anti-IF or anti-PC antibodies.

Sample size calculation

The highest reported prevalence of metformin induced vitamin B12 deficiency would be used to produce the most conservative sample size. Using local data done in Hong Kong, Wong CW et al showed that the prevalence of vitamin B12 deficiency in institutionalized elderly diabetic patients taking metformin was 53.2%.[20] By using a computerized sample size calculator with the assumptions of 5% precision, 50% prevalence and infinite population size, the estimated sample size was at least 385 in order to achieve 95% level of significance.[28] The sample size was set to be at least 400 to include anticipated subject withdrawal.

Outcomes

The primary outcome was the prevalence of vitamin B12 deficiency among patients with type 2 diabetes mellitus treated with metformin in a primary care setting. The secondary outcome was the associated factors for development of metformin induced vitamin B12 deficiency.

RESULTS

Study population

545 patients who fulfilled the inclusion criteria were randomly selected within the study period. Among the selected patients, 139 (25.5%) were excluded as shown in Figure 1. A total of 406 individuals were included in the final analysis. (Fig 1)

The demographic and clinical characteristics of the subjects were shown in Table 1. The mean age of our patients was 66.6 years in which 53.7% were female. The mean body mass index was 25.4 kg/m^2. Most of the patients were non-smokers (76.3%) and non-drinkers (83.5%). Only 1 patient (0.2%) was a vegetarian. 43 patients (10.6%) had received CSSA and 10 patients (2.5%) were on PPI.

Nearly half (49%) of the patients had diabetes for more than 10 years. Regarding the use of metformin, one-third (28.8%) of the patients were on metformin for less than 5 years, while one-third (37.2%) for 5 to 10 years, and one-third (34%) for more than 10 years. Nearly half (47%) of the patients were on 2000mg of metformin or above per day. Around two-third (60.9%) of the patients had HbA1c level of less than 7%. (Table 1)

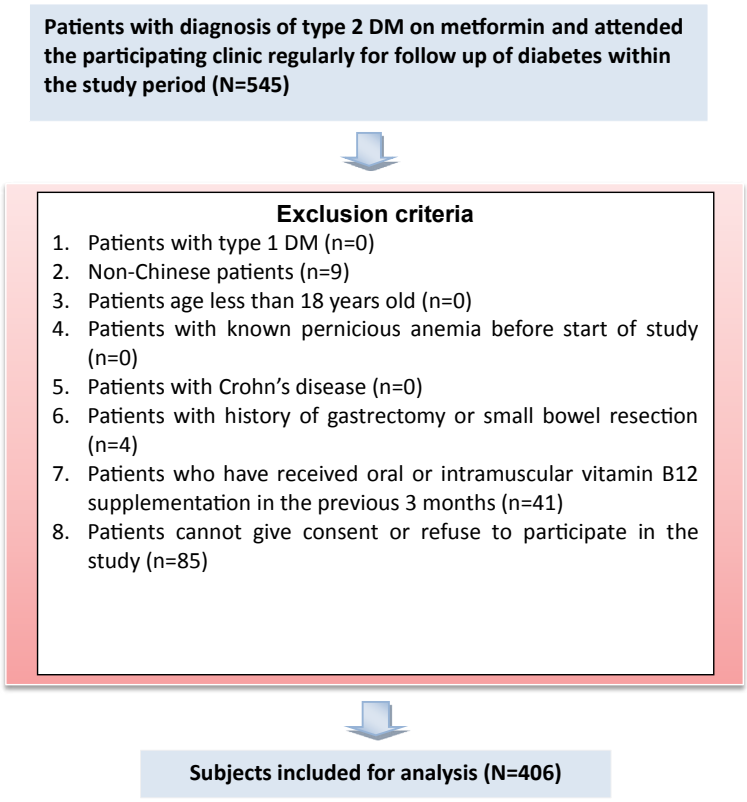

Figure 1 Flow chart on subject selection

Table 1: Demographic data and clinical characteristics of patients (N=406)

	Mean (SD)	Number (%)
Age (years)	66.6 (9.7)	
<40		1 (0.2)
40-49		15 (3.7)
50-59		74 (18.2)
60-69		168 (41.4)
70-79		105 (25.9)
≥ 80		43 (10.6)
Sex		
Male		188 (46.3)
Female		218 (53.7)
Body mass index (kg/m^2)	25.4 (4.0)	
<18.5 (underweight)		4 (1.0)
18.5-22.9 (normal)		109 (26.9)
23-24.9 (overweight)		102 (25.1)
≥25 (obesity)		191 (47.0)
Smoking status		
Non-smoker		310 (76.3)
Ex-smoker		75 (18.5)
Chronic smoker		21 (5.2)
Drinking status Non-drinker Ex-drinker Social drinker Chronic drinker		 339 (83.5) 17 (4.2) 44 (10.8) 6 (1.5)
Vegetarian Yes No		 1 (0.2) 405 (99.8)
On CSSA Yes No		 43 (10.6) 363 (89.4)
On PPI Yes No		 10 (2.5) 396 (97.5)
Duration of diabetes (years)		
<5		65 (16.0)
5-10		142 (35.0)
>10		199 (49.0)
Duration of metformin usage (years)		
<5		117 (28.8)
5-10		151 (37.2)
>10		138 (34.0)
Metformin dosage (mg/ day)		
<1000		66 (16.3)
1000 - <1500		88 (21.7)
1500 - <2000		61 (15.0)
≥ 2000		191 (47.0)
HbA1c level (%)		
<6.5		79 (19.5)
6.5-6.9		168 (41.4)
≥ 7		159 (39.1)

Abbreviations: SD: Standard Deviation, CSSA: Comprehensive Social Security Assistance, PPI: Proton Pump Inhibitor

The prevalence of vitamin B12 deficiency

135 (33.3%) patients had vitamin B12 deficiency, defined by low serum total or active vitamin B12 levels (Table 2). Among this group of patients, 104 (25.6%) patients had only low levels of total vitamin B12 but normal active vitamin B12 levels; while 2 (0.5%) patients had only low levels of active vitamin B12. 29 (7.1%) patients had low levels of both total and active vitamin B12. The only vegetarian in our study did not develop vitamin B12 deficiency.

Out of a total of 135 patients who had low serum total or active vitamin B12 levels, subsequent blood tests showed that 44 (32.6%) had positive anti-PC/ anti-IF antibodies (Table 2). 14 patients refused to come back for further blood tests. Since the proportion of patients without positive antibodies was higher than those with antibodies, these 14 patients were assumed to have metformin induced rather than pernicious anemia related vitamin B12 deficiency during statistical analysis. Thus after excluding those with possible pernicious anemia, 91 (22.4%) patients had vitamin B12 deficiency induced by metformin. (Table 2)

Univariate analysis on associated factors for metformin induced vitamin B12 deficiency

After excluding those patients with possible pernicious anemia, 362 patients were included for analysis of factors associated with metformin induced vitamin B12 deficiency. Comparing the group of patients who had vitamin B12 deficiency induced by metformin to the group with no vitamin B12 deficiency as illustrated in Tables 3 and 4, there were no significant differences for sex, age, BMI status, smoking status, drinking status, socioeconomic status, concomitant use of PPI, and presence of anemia or macrocytosis. The two groups had comparable HbA1c level (median: 6.7% versus 6.8%). On the other hand, there were significant differences for the duration of diabetes, duration of metformin usage and metformin dosage. This showed that even when patients' diabetes were in good control, the longer duration of diabetes, the longer duration of

Table 2: Prevalence of metformin induced vitamin B12 deficiency (N=406)

	Number (%)
Prevalence of vitamin B12 deficiency in all patients, with either low total or active B12 levels (N=406)	135 (33.3)
Patients with low total B12 levels only	104 (25.6)
Patients with low active B12 levels only	2 (0.5)
Patients with low total B12 *AND* active B12 levels	29 (7.1)
Subgroup analysis on the detection of APC or AIF among those with vitamin B12 deficiency (n=135) Yes No Unknown	 44 (32.6) 77 (57.0) 14 (10.4)
Prevalence of metformin induced vitamin B12 deficiency (N=406)	**91 (22.4)**

Abbreviations: APC: Anti-Parietal Cell Antibody, AIF: Anti-Intrinsic Factor Antibody

Table 3: Risk factors associated with development of metformin induced vitamin B12 deficiency: categorical variable (N=362)

Risk factors		Metformin induced vitamin B12 deficiency (n=91)		No vitamin B12 deficiency (n=271)		P-value
		Number	%	Number	%	
Sex	Male	42	46.2	130	48.0	0.764
	Female	49	53.8	141	52.0	
Smoking	Non-smoker	71	78.0	205	75.6	0.826
	Ex-smoker	15	16.5	53	19.6	
	Chronic smoker	5	5.5	13	4.8	
Drinking	Non-drinker	76	83.5	225	83.0	0.592
	Ex-drinker	5	5.5	11	4.1	
	Social drinker	10	11.0	30	11.1	
	Chronic drinker	0	0	5	1.8	
On CSSA	Yes	11	12.1	27	10.0	0.567
	No	80	87.9	244	90.0	
On PPI	Yes	1	1.1	8	3.0	0.459
	No	90	98.9	263	97.0	
Anemia	Yes	21	23.1	46	17.0	0.195
	No	70	76.9	225	83.0	
Macrocytosis	Yes	9	9.9	22	8.1	0.601
	No	82	90.1	249	91.9	

Abbreviations: CSSA: Comprehensive Social Security Assistance, PPI: Proton Pump Inhibitor

Table 4: Risk factors associated with development of metformin induced vitamin B12 deficiency: continuous variable (N=362)

Risk factors	Metformin induced vitamin B12 deficiency (n=91)		No vitamin B12 deficiency (n=271)		P-value
	Mean	SD	Mean	SD	
Age (years)	67.1	9.6	66.2	9.7	0.478
Body mass index (kg/m^2)	25.1	3.8	25.4	4.0	0.456
Hemoglobin level (Male) (g/dL)	13.9	1.2	14.1	1.4	0.448
Hemoglobin level (Female) (g/dL)	12.7	1.2	13.0	1.1	0.135
	Median		Median		P-value
Duration of diabetes (years)	12		9		**0.001**
Duration of metformin usage (years)	11		7		**<0.001**
Metformin dosage (mg)	2000		1500		**<0.001**
HbA1c level (%)	6.7		6.8		0.981
Total vitamin B12 level (pmol/L)	99.0		228.0		**<0.001**
Active vitamin B12 level (pmol/L)	52.0		123.4		**<0.001**
Mean corpuscular volume level (fL)	90.2		90.7		0.598

Abbreviation: SD: Standard Deviation

Table 5: Risk factors associated with development of metformin induced vitamin B12 deficiency: Logistic Regression (Initial model)

Risk factors	P-value	Odds Ratio	95% Confidence Interval
Age (years)	0.52	1.01	0.98 to 1.04
Sex	0.76	0.91	0.50 to 1.66
Duration of diabetes (years)	0.24	0.96	0.89 to 1.03
Duration of metformin usage (years)	0.15	1.07	0.98 to 1.17
Metformin dosage Comparing with <1000mg/day	<0.01		
1000 - <1500mg/day	0.09	2.83	0.85 to 9.36
1500 - <2000mg/day	**0.02**	**4.47**	**1.29 to 15.45**
≥ 2000mg/day	**<0.01**	**9.29**	**2.97 to 29.04**
HbA1c level (%)	0.19	0.80	0.57 to 1.12
Body mass index (kg/m^2)	0.40	0.97	0.91 to 1.04
Smoking status			
Reference: non-smoker	0.82		
Ex-smoker	0.59	0.81	0.37 to 1.76
Chronic smoker	0.63	0.74	0.22 to 2.47
Drinking status			
Reference: non-drinker	0.94		
Ex-drinker	0.63	1.36	0.39 to 4.79
Social drinker	0.75	0.87	0.37 to 2.04
Chronic drinker	1.00	<0.01	Not applicable
On CSSA	0.34	1.54	0.64 to 3.70
On PPI	0.34	0.35	0.04 to 2.99
Macrocytic anemia	0.18	3.18	0.59 to 17.23

Abbreviations: CSSA: Comprehensive Social Security Assistance, PPI: Proton Pump Inhibitor

use of metformin, and the higher metformin dosage were all associated with developing vitamin B12 deficiency. The median of total vitamin B12 levels in the vitamin B12 deficiency group was much lower than the normal group (99 pmol/L versus 228 pmol/L), and similarly for the active vitamin B12 levels (52 pmol/L versus 123 pmol/L). (Table 3, 4)

Multivariate analysis on associated factors for metformin induced vitamin B12 deficiency

For the multivariate analysis, Table 5 showed the initial model of the logistic regression. The only significant risk factor associated with developing metformin induced vitamin

Table 6: Logistic Regression (Final model, using backward method by likelihood ratio)

	P-value	Odds Ratio	95% Confidence Interval
Metformin dosage			
Comparing with <1000mg/day	<0.01		
1000 - <1500mg/day	0.09	2.78	0.85 to 9.04
1500 - <2000mg/day	**0.04**	**3.71**	**1.10 to 12.54**
≥2000mg/day	**<0.01**	**8.03**	**2.76 to 23.34**
Macrocytic anemia	0.06	4.51	0.97 to 21.02

B12 deficiency after adjusting the confounding factors was the metformin dosage. Compared with metformin dosage <1000mg/day, the odds ratio (OR) of developing vitamin B12 deficiency for 1500mg to <2000mg/day was 4.47 (95% C.I.: 1.29 to 15.45; p=0.02); and for ≥2000mg/day was 9.29 (95% C.I.: 2.97 to 29.04; p<0.01).

Further analysis using the backward method by likelihood ratio (Table 6) showed that once again, the metformin dosage was a significant risk factor for developing metformin induced vitamin B12 deficiency. Compared with metformin dosage <1000mg/day, the OR of developing vitamin B12 deficiency for 1500mg to <2000mg/day was 3.71 (95% C.I.: 1.10 to 12.54; p=0.04); and for ≥2000mg/day was 8.03 (95% C.I.: 2.76 to 23.34; p<0.01). In addition, the presence of macrocytic anemia also showed a borderline statistically significant result with OR 4.51 (95% C.l.: 0.97 to 21.02; p=0.06) (Table 5, 6)

DISCUSSION

In our study, more than one-fifth (22.4%) of patients were found to have metformin induced vitamin B12 deficiency. This result was comparable to previous published overseas studies [3-5]. When compared with other Asian countries, our prevalence was also similar to Korea (Kim J et al, 22.2%) [29] But lower than India (Kothiwale V et al, 35%) [30]. While two previous studies from Hong Kong showed a much higher prevalence (52 and 53.2% respectively) [19,20], it is important to note that they were both conducted among the geriatric population, with the mean age being 80 years and 83 years respectively. The older population is likely to be frailer with multiple comorbidities, and have a higher tendency to become malnourished.

Regarding the risk association with using metformin, our study showed that the metformin dosage was the only significant risk factor for developing vitamin B12 deficiency. Patients using a daily metformin dose of ≥2000mg had a much higher risk of developing vitamin B12 deficiency when compared with patients using low dose (<1000mg/day) metformin. Comparing our results to previous studies, Ko SH et al and Ting R et al both found that higher daily dose of metformin and longer duration of metformin usage were associated with higher risk of vitamin B12 deficiency.[13,14] In contrast, Kim J et al showed very similar results to our study that there was a correlation between vitamin B12 deficiency and higher metformin dosage but not the duration of metformin use.[29]

In conclusion, using a higher dose of metformin definitely has a higher risk of developing vitamin B12 deficiency, but in regard to the duration, further studies should be conducted to evaluate the association. Other factors like sex, age, BMI status, smoking status, drinking status, socioeconomic status, HbA1c level and the duration of diabetes all failed to show an association with metformin induced vitamin B12 deficiency in our study. This could be due to the small sample sizes of some of the factors, especially for smoking and drinking statuses. Furthermore, using only CSSA to define on one's socioeconomic status might only reflect the patients' current financial status and not be truly representable.

Out of 91 patients with metformin induced vitamin B12 deficiency, only 21 (23.1%) had anemia and only 9 (9.9%) had macrocytosis. The prevalence of anemia or macrocytosis did not differ between patients with and without vitamin B12 deficiency, and so the findings aligned with previous referenced studies.[12,13] Ko SH et al pointed out that masking of the macrocytic expression of megaloblastic anemia could be due to coexisting thalassemia, iron deficiency and chronic illness.[13] In conclusion, this stressed the importance of checking vitamin B12 levels even if there were no anemia or macrocytosis.

In contrast to previous studies which showed good concordance between the total and active vitamin B12 levels [21,23,31], our results showed that out of 135 vitamin B12 deficient patients, only around 20% of patients had both serum total and active vitamin B12 deficiencies. As aforementioned, although NICE guideline has suggested the use of active vitamin B12 assay to replace total vitamin B12 level for testing of vitamin B12 deficiency [27], total vitamin B12 level was still conventionally being used in many settings. Therefore, we have decided to define the patient as vitamin B12 deficient when either total or active vitamin B12 levels were low. However, further studies should be done to evaluate on the less than expected discordance between the 2 assays being used in this study.

Although some overseas studies found an association between the use of metformin with PPI and development of vitamin B12 deficiency [11,12], our study as well as previous local studies [14,19-20] failed to support this association. However, the results from our study should be interpreted carefully since the number of patients who were on PPI was exceptionally low and the past history of PPI use was not evaluated.

Vitamin B12 is a water-soluble vitamin which has to be absorbed through our diet. A systematic review summarized that amongst vegetarians, adults and elderly individuals with ages ranging from 18 to 97 years had a deficiency range of 0-86.5%.[32] In our study, since only 1 (0.2%) patient was a vegetarian, we could not assess the association with vitamin B12 deficiency. Nevertheless, an unbalanced diet be it vegetarian or not could still increase the risk of vitamin B12 deficiency. For future studies, a detailed dietary history should be obtained from each patient for assessment.

Limitations

There were few limitations in this study. Firstly, the cross-sectional design of our study limited the establishment of causal relationship between metformin associated vitamin B12 deficiency and the associated risk factors. To determine their causal relationship, future longitudinal studies are needed. Secondly, we had not ruled out other important gastric diseases such as atrophic gastritis which could also cause vitamin B12 deficiency. Thirdly, subjects were recruited from one General Out-patient Clinic only which limited the generalizability of our results to the whole population of Hong Kong. Fourthly, we had excluded 41 patients who were already on vitamin B12 supplement during subject selection. Theoretically they should be included in the calculation of the prevalence of metformin induced vitamin B12 deficiency, but we had excluded them as their causes of vitamin B12 deficiency could not be ascertained and we would also like to evaluate on the median of vitamin B12 levels. Lastly, there were 14 vitamin B12 deficient patients who refused to come back for further blood tests to check for pernicious anemia, but we had included them as metformin induced vitamin B12 deficiency based on the result that positive anti-IF or anti-PC antibodies were the minority. This could potentially overestimate the prevalence of metformin induced vitamin 12 deficiency.

CONCLUSION

Patients taking high dose of metformin (≥1500mg/day) should be screened for vitamin B12 deficiency periodically in primary care even if they had no anaemia or macrocytosis.

AUTHORS CONTRIBUTION

Ka-Yu Doogie Yeung was the principal investigator who wrote the manuscript.

Pang Fai Chan and Kit-Ping Loretta Lai both contributed to the protocol development of the study, the analysis and interpretation of the data. They also edited the manuscript and made a significant contribution that improved the content of the manuscript.

Man-Hei Matthew Luk contributed to the design of the study, the analysis and interpretation of the data.

David Via-Kiong Chao contributed to the protocol development, budget of the study and also edited the manuscript.

Yu-De Eudora Chow and Ching-Ching Alice Wong both contributed to the protocol development of the study and also edited the manuscript.

All 7 authors agreed to the contents of the final manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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