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Independent Effect of Vitamin B_{12} Deficiency on Hematological Status in Older Chinese Vegetarian Women

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We have examined the independent effect of vitamin B_{12} deficiency on hematological indices in older Chinese vegetarian women using a cross-sectional study design: 119 women older than 55 years who had been vegetarian for more than 3 years were studied. Fasting blood samples were taken for complete blood count, serum iron, total serum iron binding capacity, serum iron saturation, serum vitamin B_{12}, serum folate, serum methylmalonic acid levels (MMA), and renal function test. Subjects with iron deficiency (iron saturation <15%) and those with serum creatinine >150 mmol/L were excluded. The prevalence of definite vitamin B_{12} deficiency (vitamin B_{12} level < 150 pmol/L and MMA ≥ 0.4 μmol/L) was 42%. Another 32.8% had possible vitamin B_{12} deficiency (either criterion). The prevalence of iron deficiency was 10%. After exclusions, 96 subjects were further analyzed. Vitamin B_{12} deficiency defined by serum vitamin B_{12} and MMA was associated with a decrease in hemoglobin concentrations by up to 0.9 g/dL, but it was not associated with an increase in mean corpuscular volume (MCV). Serum MMA but not vitamin B_{12} levels correlated inversely with hemoglobin and platelet counts and positively with MCV, after adjustment of confounding factors. However, the percentage of subjects with anemia did not increase significantly until serum MMA became >1.0 μmol/L. In conclusion, vitamin B_{12} deficiency was associated with a significant decrease in hemoglobin concentration. However, anemia associated with vitamin B_{12} deficiency was seldom macrocytic. We recommend that older vegetarians should be given vitamin B_{12} supplements routinely. Am. J. Hematol. 70:186–190, 2002.

Key words: vegetarianism; vitamin B_{12}; anemia; aged

INTRODUCTION

Vegetarians are at risk of vitamin B_{12} and iron deficiency [1,2]. However, comparative studies showed either no difference or only minor differences in hemoglobin concentrations and mean corpuscular volumes between vegetarians and omnivores [3–5], except when there was a high prevalence of iron deficiency [6]. Macrocytosis associated with vitamin B_{12} deficiency was remarkably rare in these studies. It is possible that the high folate intake and co-existing iron deficiency in vegetarians mask the classic hematological manifestations of vitamin B_{12} deficiency. It is also possible that the degree of vitamin B_{12} deficiency among vegetarians is usually not severe enough to cause macrocytosis or anemia [7]. However, there is also evidence that older people may be more susceptible to macrocytosis with vitamin B_{12} deficiency. In a study of 56 geriatric outpatients with low serum B_{12} levels, 13 (23%) individuals had macrocytosis [8].

These studies defined vitamin B_{12} deficiency by serum vitamin B_{12} level. Recent studies had shown that up to a quarter of subjects with serum vitamin B_{12} levels as low as <100 pmol/L could have no clinical or biochemical evidence of deficiency [9]. On the other hand, 10–40% of older people within serum vitamin B_{12} levels between

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150 and 300 pmol/L had elevated serum methylmalonic acid (MMA), suggestive of tissue cobalamin deficiency [10].

We have previously reported that a group of elderly Chinese vegetarian women in Hong Kong had a 54% prevalence of subnormal vitamin B_{12} (<150 pmol/L) and 30% prevalence of anemia (hemoglobin <12 g/dL). The mean hemoglobin concentration of the vegetarians was significantly lower than that of the local non-vegetarian controls. Iron deficiency was present in 15% of the anemic cases [11]. In a large study of 3,714 patients, the prevalence of serum B_{12} level <140 pmol/L among patients with normocytosis, microcytosis, macrocytosis, with anemia, and without anemia were 5.4%, 5.2%, 9.8%, 5.2%, and 6.2%, respectively. It was concluded that no matter what screening criteria were used, a significant number of patients with cobalamin deficiency would be missed [12]. Serum MMA was not measured in these two studies; therefore one might argue whether these patients had tissue cobalamin deficiency.

We re-analyzed the data after including serum methylmalonic acid levels and excluding subjects with iron deficiency and significant renal impairment. The objective was to investigate the independent effect of varying degrees of vitamin B_{12} deficiency in the hematological status of older Chinese vegetarians.

SUBJECTS AND METHODS

Female ambulatory vegetarians older then 55 years were recruited. They had been vegetarian for more than 3 years because of either religious reasons (Taoism and Buddhism) or admission to vegetarian old-age homes. They were all self-ambulatory, but some were partially dependent in activities of daily living. Consent for blood sampling was obtained from the study subjects. The study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Overnight fasting blood samples were taken for complete blood count, serum iron, total serum iron binding capacity, serum iron saturation, serum vitamin B_{12}, serum folate, serum methylmalonic acid levels (MMA), and renal function test.

The serum samples were frozen at −20°C within 6 hr of collection. Serum cobalamin and folate concentrations were determined by a solid-phase no-boil Dualcount Radioimmunoassay. Serum MMA was analyzed by capillary gas chromatography–mass fragmentography. The intra-assay and inter-assay coefficients of variation were 3.4% and 12.8%, respectively.

Complete blood count was performed on a Maxim Cell Count analyzer (Coulter Instruments, Hialeah, FL). Total iron binding capacity and serum iron were determined on a Vitrosis autoanalyzer (Roche Diagnostics, Nutley, NJ). Other biochemical tests were carried out on a Dimension autoanalyzer (Dupont, Wilmington, DE).

Anemia was defined to be hemoglobin level <12 g/dL. Normal range of mean corpuscular volume was 80–97 fL. Lower limits of normal white cell count and platelet count were 4.0×10^9/L and 140×10^9/L, respectively. Iron deficiency was defined as iron saturation <15%. The lower limit of normal folate level was 2.1 μg/L.

The lower limit of normal serum vitamin B_{12} level by our assay was 187 ± SE 37 pmol/L; therefore we chose a level of 150 pmol/L as our cutoff point. The upper normal limit of serum MMA was 0.4 mmol/L, as suggested by Allen et al. [13]. Subjects with low vitamin B_{12} levels and raised serum MMA were considered to be definitely vitamin B_{12} deficient. Those with low vitamin B_{12} or raised MMA were considered to be possible cases of vitamin B_{12} deficiency.

Statistical Method

For the analysis of the influence of vitamin B_{12} status and hematological indices, subjects with iron deficiency or serum creatinine >150 mmol/L were excluded. The rationale for excluding patients with renal failure was that renal failure would affect the hemoglobin concentrations and serum MMA levels.

The hematological profile and baseline characteristics of the three vitamin B_{12} status groups were compared by ANOVA. The potential correlation between serum vitamin B_{12} or MMA and hematological indices was examined by correlation analysis. Univariate analysis of variance was then performed to examine the association between serum vitamin B_{12} or MMA and hematological indices, adjusting for old-age home residence, dependency level, age, iron saturation, and folate levels.

RESULTS

One hundred nineteen female vegetarian subjects participated the study. Twenty-six subjects in a vegetarian old-age home took a daily multivitamin and mineral supplement that contained 5 mg of iron but no vitamin B_{12}. There were nine missing values for iron status because of insufficient samples. There were 11 subjects (11%) with iron deficiency. If one excluded those subjects on iron supplement, the prevalence of iron deficiency was 11/89 (12.4%). Out of the 11 subjects with iron deficiency, 5 had normocytic anemia and 2 had microcytic anemia. The prevalence of definite or possible vitamin B_{12} deficiency was 89/119 (75%). No subject had subnormal serum or RBC folate levels.

After excluding subjects with iron deficiency, missing serum iron levels, and raised serum creatinine levels (3 subjects, maximum 290 mmol/L), 96 subjects were further analyzed. The percentages of raised serum MMA at vitamin B_{12} levels <150 pmol/L, between 150 and 300

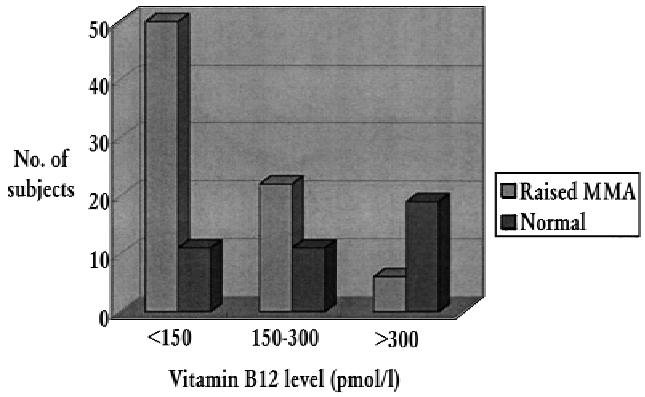

Fig. 1. Distribution of raised serum methylmalonic acid (MMA ≥ 0.4 μmol/L) levels at different serum vitamin B_{12} levels.

pmol/L, and >300 pmol/L were 80%, 67%, and 16%, respectively (Fig. 1). The characteristics and hematological profile of subjects with different vitamin B_{12} status are compared in Table I. The deficient groups were more likely to be vegans and have lower hemoglobin concentrations.

Serum MMA but not vitamin B_{12} correlated with hemoglobin concentrations (correlation coefficient −0.34, $P = 0.001$), MCV (0.31, $P = 0.003$), and platelet count (−0.20, P 0.05). Neither serum MMA nor vitamin B_{12} correlated with white cell count. On univariate analysis, adjusting for age, old-age home residence, partial dependency, iron saturation, and folate level, serum MMA correlated inversely with hemoglobin concentration and platelet count and positively with MCV ($P = 0.000$, 0.03, 0.005, respectively). Prevalence of anemia, macrocytosis, and thrombocytopenia at different levels of serum MMA is shown in Table II. There was no significant increase in anemia until serum MMA was >1.0 μmol/L (P 0.03, Chi-square test). There was no association between low vitamin B_{12} levels (defined as either <150 or <300 pmol/L) and any of the hematological indices (Chi-square test). Our study subjects did not have hypothyroidism or alcoholic liver disease, which might increase the MCV.

DISCUSSION

This study showed definite and possible vitamin B_{12} deficiency was associated with a decrease in hemoglobin concentrations (12.1 vs. 13 g/dL). However, anemia (Hb < 12 g/dL) tended to occur only when metabolic deficiency was moderately severe.

As previously shown, this group of older vegetarian Chinese women had significantly lower hemoglobin concentrations than their local omnivorous counterparts. The prevalence of iron deficiency anemia was 6.4% (7/110). This was higher than that reported in postmenopausal South Asians in the U.K. [14] and the 2.5% reported in 427 older Chinese people with low socioeconomic status in Hong Kong [15]. However, it was at the lower end of the range reported in vegetarians in Asians and Caucasians [6,16]. Although our vegetarian subjects had dietary iron intakes comparable to those of omnivores of similar age [11], they were still at risk of iron deficiency because of the presence of inhibitors of absorption of non-heme iron, e.g., fiber and phytate, from vegetable sources [17]. The majority of iron-deficient subjects were anemic. Low serum iron saturation may not distinguish iron deficiency from anemia of chronic disease; however, by excluding them from analysis, the independent effect of vitamin B_{12} could be examined. As expected, the serum folate levels of these vegetarians were high. It has been shown that high folate intake could delay the hematological manifestations of vitamin B_{12} deficiency but it remains ineffective in preventing its neurological complications [7] or a rise in metabolite (homocysteine) level [18,19]. Therefore, the association between vitamin B_{12} deficiency and hematological problems might have been attenuated in this study group.

The prevalence of vitamin B_{12} deficiency was very high in this group of vegetarians, probably because of advanced age and long years of vegetarianism. In addition, many of our older vegetarians were vegans. Even Buddhists who were allowed to drink milk seldom did so because dairy products are not popular foods among Hong Kong Chinese. Subjects in institutional care were encouraged to drink milk or nutritional supplements that contained some vitamin B_{12}, and this was associated with a lower prevalence of deficiency. Egg is good source of vitamin B_{12}, but it was not consumed frequently enough to make a difference in vitamin B_{12} status of the minority of vegetarians who did eat them.

The inverse correlation between raised serum MMA and hemoglobin was consistent and significant. The confounding effects of iron deficiency and renal failure were excluded, and other potential confounders were adjusted for. The average difference in hemoglobin concentrations between the deficient and the non-deficient subjects was up to 0.9 g/dL. This might not be sufficient to render the subjects anemic. The current recommended cutoff value of normal hemoglobin concentrations is lower in women. This sex difference was also shown in our local older population [15,20]. However, the validity of this statistical approach in defining anemia in women has recently been called into question because many women with iron deficiency were included in establishing reference ranges [21]. The Baltimore Women's Health and Aging Study showed a decreasing trend of 3-year mortality from all causes for elderly community-dwelling disabled women whose hemoglobin ranged between 12 and 14 g/dL. It was suggested that the optimal hemoglobin cutoff for anemia in older women should be >12 g/dL [22]. It is

TABLE I. Characteristics and Hematological Profile of Older Chinese Vegetarian Women With and Without Vitamin B_{12} Deficiency*

	Vitamin B_{12} deficiency						
	No	(N = 23)	Possible	(N = 28)	Definite	(N = 45)	
	Mean	SD	Mean	SD	Mean	SD	P value[b]
Age (years)	78.1	9.1	80.2	7.2	78.0	6.8	NS
OAH	13	56.5%	18	64.3%	23	51.1%	NS
Partially dependent	21	8.7%	5	17.9%	2	4.4%	NS
Milk intake[a]	19	82.6%	20	71.5%	18	40%	0.001
Egg intake	7	30.4%	9	32.1%	6	13.3%	NS
Vegetarian duration (yr)	35.3	15.4	42.5	22.1	33.4	17.6	NS
Iron saturation (%)	25.9	8.2	26.0	7.0	25.5	7.0	NS
Vitamin B_{12} (pmol/L)	489	420	273	316	87	29	0.000
MMA (μmol/L)	0.2	0.1	0.8	0.6	1.5	2.0	0.002
Folate (μmol/L)	49.0	9.2	46.3	9.0	45.4	10.5	NS
HB (g/dL)	13.0	1.3	12.1	1.0	12.4	1.2	0.03
MCV (fL)	90.6	9.6	88.8	8.2	90.2	9.0	NS
WBC ($\times 10^9$/L)	6.3	1.7	7.1	2.3	6.6	1.6	NS
PLT ($\times 10^9$/L)	221	57	226	66	206	62	NS

*Abbreviations: OAH, old age home residence; MMA, serum methylmalonic acid; folate, serum folate; HB, hemoglobin; MCV, mean corpuscular volume; WBC, white cell count; PLT, platelet count; NS, not significant.
[a]Nutritional supplements included.
[b]ANOVA for continuous data; Kruskal–Wallis test for nominal data.

TABLE II. Percentages of Abnormal Hematological Indices in Difference Degrees of Metabolic Vitamin B_{12} Deficiency*

	Serum methylmalonic acid level		
	Normal <0.4 μmol/L	Borderline raised 0.4–1.0 μmol/L	Raised >1.0 μmol/L
Anemia	3 (9.4%)	9 (22.5%)	12 (48.0%)
Macrocytosis	2 (6.5%)	3 (7.5%)	4 (16.0%)
Thrombocytopenia	1 (3.1%)	1 (2.5%)	4 (16.0%)
Total	31 (100%)	40 (100%)	25 (100%)

*Definitions: anemia, haemoglobin < 12g/dL; macrocytosis, mean corpuscular volume >97 fL; thrombocytopenia, platelet count <140 × 10^9/L.

therefore likely that the generally lower hemoglobin concentrations of our older vegetarians are detrimental to their health.

The lack of correlation between vitamin B_{12} levels and hematological indices was consistent with other studies, one of which involved Hong Kong Chinese subjects [12,23]. This confirmed that hematological indices could not be used to guide vitamin B_{12} screening. Serum MMA was considered a more specific indicator of tissue cobalamin deficiency [24]; 67% and 16% of our study subjects with serum vitamin B_{12} between 150 and 300 pmol/L and >300 pmol/L had raised serum MMA, respectively, therefore arbitrarily choosing a particular level as cutoff point may miss out a number of patients with tissue cobalamin deficiency. Serum holotranscobalamin II level correlates well with intracellular vitamin B_{12} and better delineates early tissue cobalamin deficiency [25]. Measuring serum holotranscobalamin in our subjects will be of interest in a future study.

Vitamin B_{12} deficiency should therefore be considered in all cases of anemia in old age, especially as multiple deficiencies of hematinics are not uncommon [26].

Vitamin B_{12} deficiency is a recognized cause of pancytopenia. In one study from northwest India, 80% of patients with nutritional megaloblastic anemia had thrombocytopenia [27]. Our study demonstrated a significant but weak negative association between elevated MMA and platelet counts. It was unlikely to be clinically significant.

We conclude that vitamin B_{12} deficiency is associated with a significant decrease in hemoglobin concentrations, but anemia occurred in the moderately severe cases with MMA >1 mol/L. Because macrocytosis was seldom present, MCV should not be relied upon to distinguish vitamin B_{12}-related anemia from other causes. In view of the high prevalence (75%) of vitamin B_{12} deficiency and the limitations of serum vitamin B_{12} in detecting deficiency states, we recommend that older vegetarians be given vitamin B_{12} supplements routinely, preferably with a preparation that is effective even for people with pernicious anemia and food-bound vitamin B_{12} malabsorption [28]. A strong case has already been made for a 100 μg vitamin B_{12} supplement for everyone over the age of 50 to prevent loss of recent memory in elderly [29]. Apart from preventing subtle neurological damages that may precede the development of anemia [28] and hyperhomocysteinemia, a consequential increase in hemoglobin concentrations may confer significant health benefits to these older people.

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