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Prevalence of vitamin B_{12} depletion and deficiency in Liechtenstein

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Abstract

Objective: Data about vitamin B_{12} (B_{12}) deficiency in the general population are scarce. The present study was performed to determine the prevalence of B_{12} deficiency in the general population of the Principality of Liechtenstein, as well as to identify sub-populations potentially at high risk.
Design: Retrospective study.
Setting: Ambulatory setting, population of the Principality of Liechtenstein.
Subjects: Seven thousand four hundred and twenty-four patients seeking medical attention whose serum samples were referred for routine work-up in an ambulatory setting were consecutively enrolled. Serum total B_{12} was determined in all patients in this cohort. In addition, for a subgroup of 1328 patients, serum holotranscobalamin was also measured. Prevalence of B_{12} deficiency was calculated. Further, multivariate logistical regression models were applied to identify covariates independently associated with B_{12} deficiency and depletion.
Results: Nearly 8% of the general population was suffering from either B_{12} depletion or deficiency. The ratio between B_{12} depletion and deficiency was 2:1 for all age ranges. Pathological changes were detected predominantly in older people. Female gender was a significant predictor of B_{12} depletion. In the cohort, nearly 40% exhibited either depletion or deficiency of B_{12}.
Conclusions: B_{12} depletion and deficiency are common in Liechtenstein, a Central European country. The measurement of biochemical markers represents a cost-efficient and valid assessment of the B_{12} state. When a deficiency of B_{12} is diagnosed at an early stage, many cases can be treated or prevented, with beneficial effects on individual outcomes and subsequent potential reductions in health-care costs.

Keywords
Vitamin B_{12}
Vitamin B_{12} deficiency
Prevalence
Serum total vitamin B_{12}
Holotranscobalamin

Vitamin B_{12} (B_{12}, cobalamin) is a water-soluble vitamin and an essential nutrient that normally must be obtained from the diet. Metabolically, it is essential for two reactions catalysed by the enzymes methionine synthase and L-methyl-malonyl-coenzyme A mutase. The daily recommended daily intake of B_{12} for adults is 2 μg[1]. In healthy individuals, nutritional B_{12} deficiency is unusual because total body stores in adults are about 2500 μg and daily turnover is slow[2], meaning that reserves generally remain for up to 10 years[3]. B_{12} deficiency can have many causes, such as nutritional habits (strict vegetarian and vegan diets: practice of abstaining from use of animal products), intestinal malabsorption (i.e. gastritis, state after total gastrectomy), use of proton pump inhibitors and elevated requirements (hyperthyroidism)[2].

Severe and persistent B_{12} deficiency has relevant adverse effects on clinical condition, namely haematological, neurological, neuropsychiatric and metabolic dysfunctions (i.e. methyl-malonyl-coenzyme A acidosis, hyperhomocysteinaemia)[3–5]. Mild B_{12} deficiency normally does not provoke clinical symptoms, but can be diagnosed by measurement of blood markers. The clinical laboratory parameters available to diagnose B_{12} deficiency are serum total B_{12}, transcobalamin-bound B_{12} (holotranscobalamin, HoloTC: active fraction of B_{12}), plasma homocysteine (Hcy) and methylmalonic acid (MMA)[6]. The metabolites Hcy and MMA can be used as indicators of B_{12} deficiency, but many factors other than

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B_{12} deficiency (e.g. renal failure) can increase Hcy and MMA. Furthermore, measuring MMA is complicated and expensive, requiring HPLC or GC–MS[7]. Thus algorithms for laboratory diagnosis of a B_{12}-deficient status recommend the initial measurement of B_{12} and HoloTC. Clarke *et al.* state that HoloTC has better diagnostic accuracy than B_{12} (77 % *v.* 73 %) and that the diagnostic utility is superior in the overall population as well as in patients with renal impairment[8].

B_{12} deficiency is characterized by low serum concentrations of total B_{12} (<148 pmol/l) and HoloTC (<35 pg/l), whereas depletion shows total B_{12} within the grey zone (148–221 pmol/l) and HoloTC lower than the cut-off[4,8,9]. To date, an internationally valid consensus as to the definition of B_{12} deficiency has not been established, since different thresholds have been used[9]. The transition from B_{12} depletion to deficiency is fluid. The early diagnosis of a deficient status is essential because simple B_{12} supplementation may reverse clinical symptoms.

B_{12} deficiency is prevalent primarily in elderly people, children and women of reproductive age, with prevalences ranging from 10 to 40 %[4,8,10–13]. In general, no relationship between B_{12} status and geographic distribution of the population can be claimed[10]. The condition has the potential to be a worldwide public health problem.

The aim of the present study was to investigate the prevalence of B_{12} depletion and deficiency based on serum total B_{12} and HoloTC concentrations in a representative population of Liechtenstein. Our secondary aim was to determine factors associated with depletion and deficiency.

Materials and methods

Study population

The current retrospective study was carried out in the resident population of the Principality of Liechtenstein, without age restrictions. The study period ranged from January 2000 until December 2007. Within the study period, the population of Liechtenstein averaged 35 168 permanent residents of nearly exclusively Caucasian origin, as described elsewhere[14]. Corrected for migration and deaths, the reference population totals 38 839.

Serum samples of 7424 consecutive patients from child age to advanced age seeking medical attention by their physicians, referred for routine laboratory work-up in an ambulatory setting, were included in the study. Out of them, a subgroup of 1328 patients was also evaluated.

Hospitalized patients were excluded from the study. In the case of multiple determinations in the same individual, only the lowest value was kept in the database and used for further analysis.

Laboratory methods

Venous blood samples were drawn from all individuals in fasting or non-fasting state into Vacutainer tubes (BD Systems, Basel, Switzerland) or Sarstedt Monovette tubes (Sarstedt, Sevelen, Switzerland). The samples were referred to the Liechtenstein central laboratory. Serum total B_{12} was measured within 24 h after venepuncture. For measurement of total B_{12} concentrations, a competitive-binding immunoenzymatic assay employing chemiluminescence was used (Access Vitamin B_{12}, run on two different analysers, Access2 and Unicel DxI800 instruments (Beckman Coulter, Nyon, Switzerland), whose agreement was previously compared).

In a subgroup of 1328 patients investigated during 2007, also HoloTC levels were measured on the Abbott AxSYM® immunochemical automated analyser (Abbott Diagnostics, Baar, Switzerland). The between-day CV, as evaluated by commercially available control materials, were 4·5 % (at 242 pmol/l), 5·5 % (at 360 pmol/l) and 6·3 % (at 911 pmol/l) for total B_{12} and 8·7 % (at 21 pmol/l) and 9·7 % (at 52 pmol/l) for HoloTC.

According to the country's validated laboratory reference values, the cut-off point for B_{12} deficiency was defined as serum level of total B_{12} <125 pmol/l[15]. The cut-off point for B_{12} depletion was defined as a serum level of 125–300 pmol/l for total B_{12} and a serum level of <35 pg/l for HoloTC[9].

Statistical analysis

The proportion of individuals with a B_{12} measurement among the general population was assessed across different age strata by using the national census data controlled for cases of migration and deaths. The prevalence of individuals with B_{12} depletion or deficiency was calculated within the cohort. Further, the prevalence of these individuals among the general population was determined by using the adjusted national census data. Differences between proportions were assessed with the χ^2 test. Finally, a logistic regression model was applied in order to detect associations between demographic factors such as age and gender and the presence of B_{12} deficiency. A P value less than 0·05 was considered statistically significant.

Results

A total of 7424 patients who sought medical attention were included in the study (ambulatory setting). This cohort comprised 19·1 % of the country's entire population. The baseline characteristics of the study cohort are given in Table 1. Mean serum total B_{12} levels fluctuated from 199 to 226 pmol/l between the different years.

Remarkably, only 12·4 % of the cohort had a serum total B_{12} level in a reference interval where B_{12} deficiency is unlikely (>300 pmol/l); 74·2 % of the cohort were within the grey zone (125–300 pmol/l), exhibiting B_{12} depletion, while 13·4 % of the cohort showed evidence of B_{12} deficiency (<125 pmol/l). In the subgroup of participants with simultaneous total B_{12} and HoloTC determination,

Table 1 Baseline characteristics of the study populations; ambulatory setting, Principality of Liechtenstein, January 2000–December 2007

	Total cohort			Subgroup with HoloTC measurement		
Variable	*n*	%		*n*	%	
No. of participants	7424	100		1328	100	
Gender						
Female	4915	66·2		884	66·6	
Male	2512	33·8		444	33·4	
	Mean	SD	Range	Mean	SD	Range
Age (years)	48	19	1–101	49	18	6–99
Mean serum total B_{12} (pmol/l)	230	130	11–1254	208	112	25–1144
Mean serum HoloTC (pmol/l)	Not measured	–	–	45	31	3–624

HoloTC, holotranscobalamin; B_{12}, vitamin B_{12}.

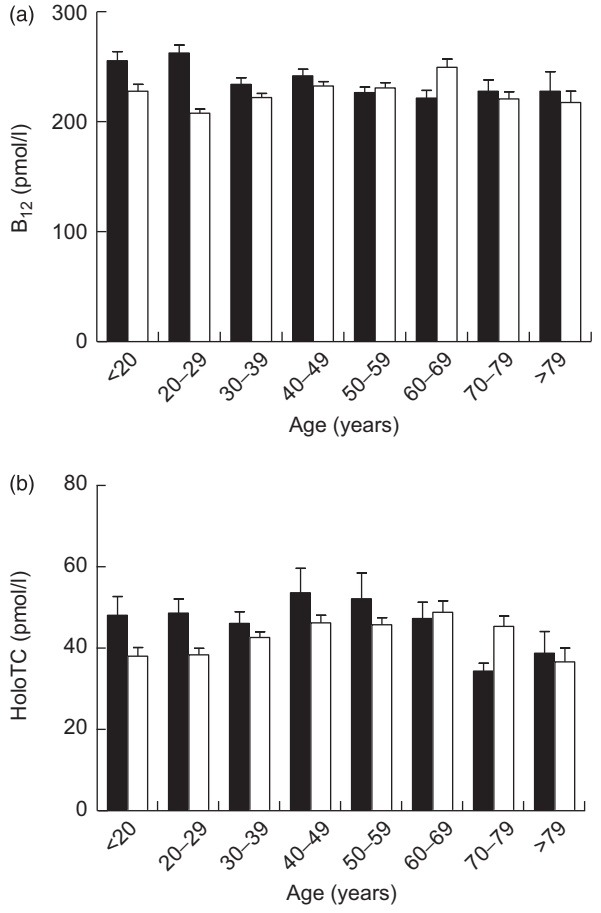

Fig. 1 Serum levels of (a) total vitamin B_{12} (B_{12}) in the study cohort (*n* 7424) and (b) holotranscobalamin (HoloTC) in a subgroup of the cohort (*n* 1328), stratified according to age and gender (■, male; □, female); ambulatory setting, Principality of Liechtenstein, January 2000–December 2007. Values are means with their standard errors represented by vertical bars

B_{12} depletion was seen in 26·4 % (i.e. B_{12} grey zone together with HoloTC <35 pmol/l). The mean total B_{12} levels and the mean HoloTC levels across the different age/gender strata are shown in Fig. 1.

Table 2 Prevalence of B_{12} depletion and deficiency in the cohort and the general population of Liechtenstein

	Cohort	Population
B_{12} depletion		
%	26·4	5·1
n/n_{cohort}	524/1328	
B_{12} deficiency		
%	13·4	2·6
n/n_{cohort} or $n_{population}$	994/7424	994/38 839

B_{12}, vitamin B_{12}; HoloTC, holotranscobalamin.
Serum total B_{12} was determined in all individuals of the cohort (*n* 7424). B_{12} depletion was measured by total B_{12} and HoloTC assays; the cohort with concomitant HoloTC determination comprised 1328 individuals. The prevalence of B_{12} depletion in the general population was extrapolated from the ratio between individuals with B_{12} depletion and B_{12} deficiency in the cohort (2:1).

The ratio between the prevalence of B_{12} deficiency and depletion was investigated in both the general population and the study cohort (Table 2). Population prevalence of B_{12} deficiency was obtained by calculating the ratio of individuals with B_{12} deficiency among the reference population of 38 839. Population prevalence of B_{12} depletion was extrapolated from the ratio between individuals with B_{12} depletion and B_{12} deficiency in the study cohort (2:1). Taking these findings into account allowed estimation of the prevalence of B_{12} depletion and deficiency among the general population at nearly 8 % (Table 2).

Stratifying the cohort with regard to gender and age showed that the prevalence of B_{12} deficiency was significantly higher in females than in males (14·85 % *v.* 10·51 %, respectively, $P < 0{\cdot}001$) and in persons aged 50 years and older than in those younger than 50 years (15·47 % *v.* 11·79 %, respectively, $P < 0{\cdot}001$; Fig. 2). Interestingly, there was a bimodal distribution of the prevalence within females, with a first peak at childbearing age. The subgroup having both total B_{12} and HoloTC measurements paralleled these findings, but demonstrated higher prevalence among all age groups (Fig. 3). Surprisingly, a remarkable B_{12}-deficient status was already seen in children.

Further calculating the prevalence of B_{12} deficiency within the general population revealed characteristics

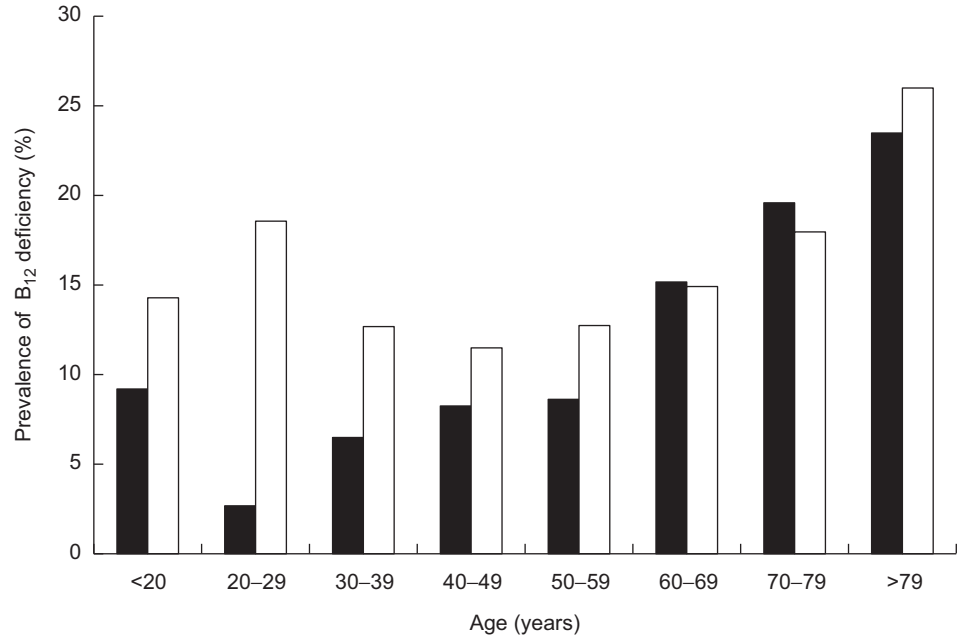

Fig. 2 Prevalence of vitamin B_{12} (B_{12}) deficiency in the study cohort (*n* 7424), stratified according to age and gender (■, male; □, female); ambulatory setting, Principality of Liechtenstein, January 2000–December 2007

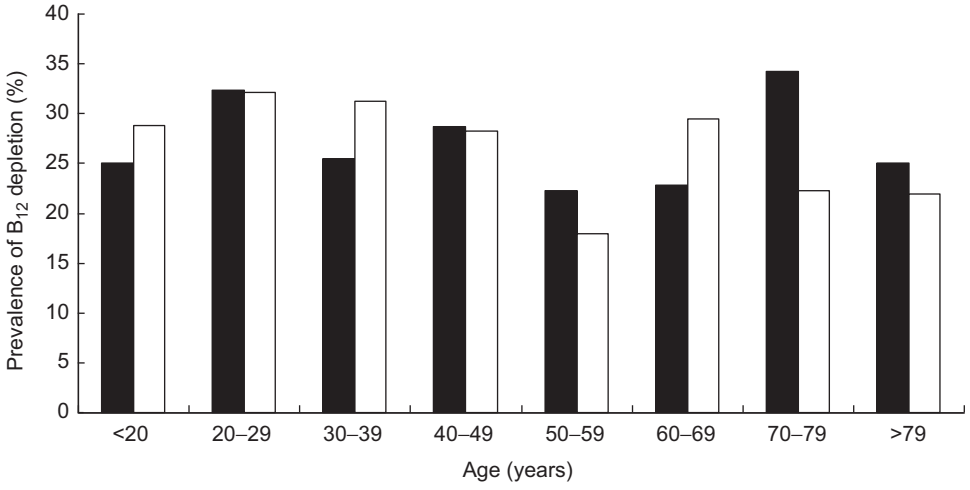

Fig. 3 Prevalence of vitamin B_{12} (B_{12}) depletion (measured by total B_{12} and holotranscobalamin assays) in a subgroup of the cohort (*n* 1328), stratified according to age and gender (■, male; □, female); ambulatory setting, Principality of Liechtenstein, January 2000–December 2007

similar to those within the cohort: older persons and females suffer more often from B_{12} deficiency (Fig. 4).

Finally, a logistic regression model with age, gender and the interaction between age and gender as predictors of B_{12} deficiency found that age (OR = 1·32; 95 % CI 1·23, 1·43) and female gender (OR = 5·81; 95 % CI 3·41, 9·89) were significant predictors of the presence of B_{12}-deficient status. Interestingly, the interaction between female gender and age was also significant (OR = 0·80; 95 % CI 0·73, 0·87), indicating that the influence of age on the frequency of B_{12} deficiency is stronger in women than in men.

Discussion

In the present retrospective study we found that nearly 20 % of the population had a clinical suspicion of B_{12} deficiency. About 40 % of the cohort had biochemical evidence of impaired B_{12} serum levels (26·4 % depleted and 13·4 % deficient). Within the general population of Liechtenstein, B_{12} deficiency is encountered in 2·6 %, whereas the prevalence of B_{12} depletion can be estimated at 5·1 %.

Comparing our data with other studies such as the Framingham Study[11], which described 12 % of elderly

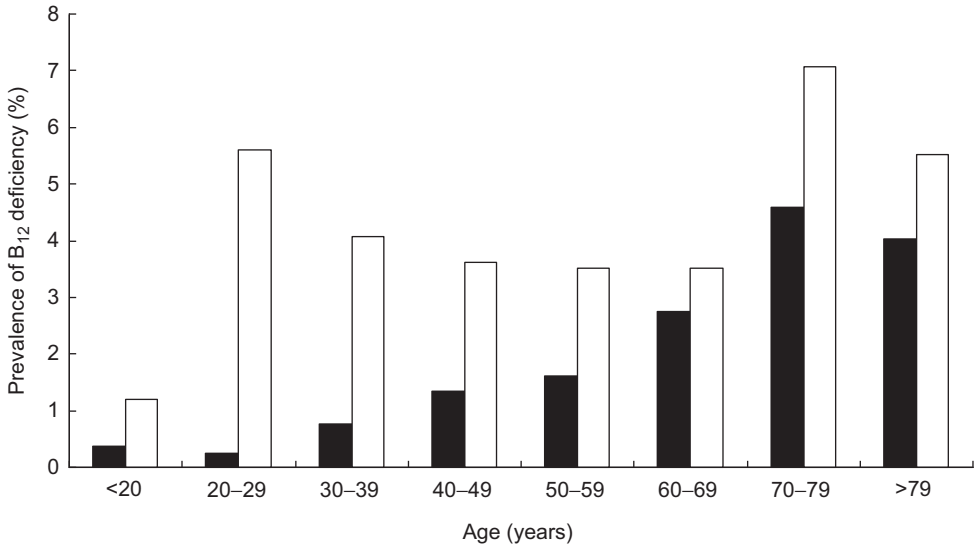

Fig. 4 Prevalence of vitamin B_{12} (B_{12}) deficiency in the general population of Liechtenstein, stratified according to age and gender (■, male; □, female)

people as suffering from B_{12} deficiency, we have to keep in mind that we were able to consecutively determine B_{12} status in persons with clinical suspicion of B_{12} deficiency within one entire country. Other studies have examined B_{12} status in individuals randomly selected from the population[10,16,17]. Allen showed in a US sub-population of individuals aged ≥60 years that the prevalence of B_{12} deficiency was 6% and the prevalence of B_{12} depletion (marginal B_{12} status, HoloTC not assessed) was about 20%[4]. According to Clarke *et al.*, in approximately 5–20% of elderly people a B_{12} deficiency remains undiagnosed[8]. The prevalence of subclinical functional B_{12} deficiency in the general population is higher than expected[7].

There are hardly any population-wide studies about B_{12} depletion or deficiency. The sub-populations examined mainly are elderly people (as mentioned above), children and women of reproductive age. Children are of special interest as early B_{12} deficiency leads to impaired brain development and a higher risk of depression as an adult[18]. Our study shows that B_{12}-deficient status occurs at all ages, showing one peak in the third decade and another in advanced age (from age 70 years onwards). On the one hand, the capacity to absorb B_{12} from food decreases in older people (i.e. atrophic gastritis, intestinal dysfunction), consequently leading to a malabsorption syndrome[19,20]. On the other hand, the principal reason for B_{12} malabsorption is the pharmacological decrease in acid secretion in the stomach, causing an impairment of protein-bound B_{12} absorption[2]. Drugs that decrease acid secretion comprise 3·9% of all administered drugs in Liechtenstein (Mag. Stefan Tomaselli, Liechtenstein Office of Public Health, personal communication). However, in our database, a link between antacid use and serum total B_{12} concentration in an individual cannot be drawn. Accordingly, we cannot provide evidence on the epidemiological importance of antacid use as a cause of B_{12} deficiency.

Among young people in Madrid, Gil *et al.* appraised 4·8% as being deficient, with males more likely to be deficient than females, whereas our data show a significantly higher prevalence among girls within a similar percentage of affected persons[21]. This fact could be explained by the fact that in Central European countries girls show a higher rate of unhealthy dietary habits than boys[22–25].

Refsum *et al.* showed that total B_{12} and HoloTC concentrations were lower in women than in man, and they increased with age[12]. Further analyses in that study revealed the age effect to be limited to women, and the gender differences were confined to those aged ≤45 years. In women ≤45 years of age, there was a complete shift of the HoloTC distribution towards lower concentrations of ~20%[12]. Would that suggest gender as a significant predictor for B_{12} deficiency? In our study about 5% of the females of this age showed a B_{12}-deficient status. B_{12} deficiency among females of reproductive age has an important impact, as it can cause infertility and abortion. Additionally, the fetus may suffer neural tube defects and prematurity[26].

Data about B_{12} deficiency and/or depletion in men are scarce. We could not identify a single study concentrating on male individuals. In general they are mentioned among either the elderly or subgroups (i.e. alcoholics, post-gastrectomy state). In our trial men less often demonstrated a deficient B_{12} status, but in association with age they had a more pronounced risk of being affected (OR = 0·8). In our cohort, males over the age of 80 years suffered even more often from B_{12} impairment than women.

HoloTC is known to be a more sensitive and specific marker than total B_{12}, especially for subclinical functional

B_{12} deficiency and depletion[27]. In this context it should be kept in mind that, remarkably, B_{12} depletion occurs twice as often as B_{12} deficiency. To our knowledge there are no published studies discussing this issue. The problem in comparing different studies is that there are no internationally agreed-upon reference laboratory values for the stratification of B_{12} deficiency and there is no widely accepted agreement about the therapeutic implications of B_{12} depletion. Patients showing manifest B_{12} deficiency have to be treated immediately after diagnosis, as some clinical symptoms can be reversed[5].

The main limitation of our study concerns the lack of performance of additional laboratory tests such as Hcy or MMA. Both are comparably expensive and laboratory-intensive tests. On the other hand, MMA concentration is considered to be the most specific and sensitive parameter for diagnosis of B_{12} deficiency[27]. Furthermore, we did not randomly analyse the population of Liechtenstein, either with regard to subgroups like pregnant women or individuals affected by metabolic disease. In addition, we did not assess the health status and the illness for which medical attention was sought.

Conclusions

B_{12} deficiency is a frequent finding in a Central European population (2·6 %), and B_{12} depletion is found twice as often. Female gender and age are independent, significant predictive factors of a B_{12}-deficient state, and regular monitoring of B_{12} status is recommended for them. Current considerations for public health interventions to prevent B_{12}-associated pathologies in vulnerable sub-populations are still under debate[28].

The measurement of total B_{12} and HoloTC concentrations are two valid and easily performed parameters for the detection of B_{12} deficiency. Early recognition of subclinical depletion of B_{12} is essential, and treatment of the deficiency is imperative, as symptoms can be reversed at an early phase and may also be preventable. Diagnosis of B_{12} status is important, uncomplicated, and can lead to simple treatment, preventing major disabilities.

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