Vitamin B12 (Cobalamin) Deficiency in Overt and Subclinical Primary Hypothyroidism

Clinical Medicine Insights:
Endocrinology and Diabetes
Volume 15: 1–8

DOI: 10.1177/11795514221086634

Mohamed Aon[1], Sherif Taha[1,2], Khaled Mahfouz[2], Mohamed M Ibrahim[3,4] and Ahmed H Aoun[5]

[1]Department of Internal Medicine, Faculty of Medicine, Cairo University, Giza, Egypt. [2]Department of Internal Medicine, Jahra Hospital, Jahra, Kuwait. [3]Department of Clinical Pharmacology, Jahra Hospital, Jahra, Kuwait. [4]Department of Clinical Pharmacology, 6th October University, 6th October city, Egypt. [5]Department of Pediatrics, Faculty of Medicine, Cairo University, Giza, Egypt.

ABSTRACT

BACKGROUND: B12 (cobalamin) deficiency has been reported in hypothyroid patients with variable prevalence rates thus routine screening of hypothyroid patients was recommended by some and discouraged by others. We aimed to assess the prevalence of B12 deficiency among hypothyroid patients and to evaluate for pernicious anemia and celiac disease as etiologies.

METHODS: A total 133 patients were included. Thyroid hormones and thyroid peroxidase (TPO) autoantibodies were measured. Serum B12 was measured and if deficient, intrinsic factor antibodies (IFAB) and tissue transglutaminase (tTG) antibodies were evaluated.

RESULTS: Our study included 45 patients with overt hypothyroidism (OH), 48 patients with subclinical hypothyroidism (SCH), and 40 patients as controls. Mean age was 34.3 years and 82% were females. TPO antibodies were positive in 73.5% of OH and 51.1% of SCH patients. B12 deficiency was detected in 33.3%, 47.9%, and 37.5% of OH, SCH, and controls, respectively with no significant difference ($P = .334$). Borderline-to-low B12 level was more prevalent in the OH and the SCH groups compared to controls (68.9%, 85.4%, and 57.5%, respectively; $P = .014$). Among B12-deficient hypothyroid patients, 7.5% had positive IFAB and 13.3% had positive tTG antibodies. We did not find a significant association of TPO positivity and B12 deficiency (OR, 0.69; 95% CI 0.3-1.57; $P = .147$).

CONCLUSION: We did not find a higher prevalence of B12 deficiency among hypothyroid patients nor an association with TPO positivity. Borderline B12 levels were more prevalent among hypothyroid patients.

KEYWORDS: Vitamin B12 deficiency, hypothyroidism, subclinical hypothyroidism, pernicious anemia, celiac disease

RECEIVED: November 30, 2021. **ACCEPTED:** February 22, 2022.

TYPE: Original Article

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Mohamed Aon, Department of Internal Medicine, Faculty of Medicine, Cairo University , Gamaet El Qahera Street, Giza, 12613, Egypt. Email: maon2004@cu.edu.eg

Introduction

Vitamin B12 (also known as cobalamin) is found naturally in animal foods or artificially in fortified cereals.[1] Cobalamin has a complex mechanism of absorption in the terminal ileum that depends on the intrinsic factor (a secretory product of the gastric parietal cells) and eventually releases cobalamin in the blood linked to the plasma binding protein, transcobalamin.[2] The prevalence of B12 deficiency varies depending on the level used to define deficiency and the populations studied. In developed countries, vitamin B12 deficiency (serum B12 level <148 pmol/L) increases with age from 3% in the younger population to reach 10% in the elderly. Borderline B12 levels or subclinical cobalamin deficiency (SCCD), which is defined as a serum B12 level between 148 and 221 pmol/L, is reported in 20% of the elderly population. In developing countries, the prevalence of low and borderline B12 levels is elevated approaching 70% in adults.[3] Furthermore, detection of B12 deficiency depends on the diagnostic strategy[4] and the type of B12 assay used in the measurement.[5]

In general, poor dietary intake and malabsorption conditions for example, pernicious anemia (PA), are the most common causes of B12 deficiency. The wrongheaded immune response in PA is directed against the gastric parietal cells and the gastric H/K–ATPase resulting in the deficiency of the intrinsic factor and achlorhydria.[2] Vitamin B12 deficiency in PA is not only caused by the loss of the intrinsic factor but is also due to the associated achlorhydria because gastric acid is needed to release cobalamin from its dietary sources.[6]

Primary hypothyroidism is a disease of the thyroid gland that results in a reduction of the blood levels of the thyroid hormones (thyroxine [T4] and triiodothyronine [T3]) and a subsequent compensatory rise in the thyroid-stimulating hormone (TSH) levels. Hypothyroidism can be either overt (OH), with elevated TSH and low free T4 levels, or subclinical (SCH), with a normal free T4 level despite the elevated TSH level.[7] Hypothyroidism affects 3% to 5% of adults and is more common in women and the elderly.[8] In iodine-sufficient regions, autoimmune thyroiditis is the most common cause of primary hypothyroidism, and it is usually associated with the antithyroid peroxidase (TPO) and the antithyroglobulin (TG) autoantibodies.[9]

The prevalence of B12 deficiency is 10% to 40% among hypothyroid patients.[10] Previous studies showed that 5% to 10% of patients with primary hypothyroidism have PA, whereas

24% of PA patients have thyroid disease.[11,12] The link between hypothyroidism and PA is related to the common autoimmune etiology of both disorders. In addition to PA, hypothyroid patients may have other abnormalities that may cause vitamin B12 deficiency such as inadequate dietary intake or decreased intestinal absorption due to slow gut motility and bacterial overgrowth.[13] Another condition that may coexist with hypothyroidism and can cause B12 deficiency is celiac disease which is an autoimmune disease of the gut that occurs in genetically susceptible individuals due to gluten sensitivity. Twenty-six percent of celiac disease patients have autoimmune thyroid disease (AITD).[14] Low serum level of vitamin B12 is seen in about 40% of untreated celiac disease patients.[15]

Both B12 deficiency and hypothyroidism can present with symptoms such as depression, memory impairment, dementia, fatigue, numbness, and paresthesia. Due to the non-specificity of symptoms, B12 deficiency may be overlooked in hypothyroid patients.[16] The early recognition and appropriate treatment of B12 deficiency in hypothyroid patients are crucial because it is a reversible cause of peripheral neuropathy (PN), myelopathy, cognitive defects, anemia, and pancytopenia.[2] However, the significance of B12 deficiency in hypothyroidism and the need to screen hypothyroid patients with serum B12 level measurement is a subject of controversy. Some studies reported a prevalence of B12 deficiency among hypothyroid patients similar to euthyroid patients,[11,17,18] while other studies reported a high prevalence of B12 deficiency among hypothyroid patients.[12,13] Moreover, the research on SCH and vitamin B12 deficiency is limited. This study aims to investigate the hypothesis that there is a higher prevalence of vitamin B12 deficiency among patients with primary hypothyroidism (OH and SCH) compared to euthyroid persons.

Materials and Methods

Study design and sample size

Our study was a cross-sectional study. Patients with primary hypothyroidism (OH or SCH) referred from primary care the to Jahra Hospital Medical Clinic, between January 2019 and October 2019, were included. Patients with no history of hypothyroidism referred from the primary care to the same clinic were included as a control group. Assuming a prevalence of B12 deficiency among normal population and patients with hypothyroidism of 3% and 25%, respectively,[17,19] we aimed for a sample size of 40 in each group[20] to achieve a study power of 80% and a confidence level of 95%.

Inclusion and exclusion criteria

Patients with past medical history or new onset of primary hypothyroidism (OH or SCH) with or without thyroid replacement therapy were included in the study. Patients who had a history of secondary hypothyroidism, hyperthyroidism, thyroidectomy, radioactive iodine treatment, or hypothyroidism related to drugs for example, amiodarone were excluded. Patients who were alcoholic, vegetarian, had a history of previous gastrointestinal surgery, pancreatic insufficiency, receiving B12 supplementation, long-term use of metformin or proton pump inhibitors (PPI), inflammatory bowel disease (IBD), or celiac disease were excluded. Pregnant females were excluded from the study.

Study variables and measurements

Data collected included demographic features, duration of illness, the dose of thyroid replacement therapy, comorbidities, concomitant drugs used, clinical features (numbness, paresthesia, memory impairment, mood changes, impaired peripheral sensation, reflexes, pallor, and glossitis). Thyroid function tests (TSH and free T4), thyroid antibodies (TPO antibodies), Hemoglobin (Hb), mean corpuscular volume (MCV), and serum B12 levels were measured. TSH, free T4, and B12 were measured using the chemiluminescent immunoassay method (DXI800, Beckman Coulter Inc., USA) and TPO antibodies were measured using the electrochemiluminescent method (cobas6000, Roche Inc., Switzerland). In patients with vitamin B12 deficiency, anti-tissue transglutaminase (tTG) antibodies and anti-intrinsic factor antibodies (IFAB) were measured. IFAB were measured using immunoassay processor, DotDiver2.0, Generic Assays, Germany.

Operational definitions

Vitamin B12 deficiency was defined as a serum level <133 pmol/L according to the manufacturer's reference range while borderline B12 level was diagnosed if serum level was 133 to 221 pmol/L.[3,21] Anemia was defined as Hb level <120 g/L in females and <130 g/L in males.[22] Iron deficiency was defined as a transferrin saturation <20% and/or ferritin <25 ng/mL.[23] TPO antibodies were considered positive if >34 IU/mL according to the manufacturer's reference range (0-34 IU/mL).

Statistical analysis

Validated data were tabulated, entered, and analyzed using Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Qualitative data were expressed as frequencies and percentages. Chi-square $\chi 2$ test was used to assess the impact of different variables on the primary outcome "B12 deficiency." Normally distributed quantitative data were expressed as mean ± SD (standard deviation). ANOVA test was used for 3 group comparisons of normally distributed quantitative variables. Non normally distributed quantitative data were expressed as median and interquartile range (IQR). Kruskal-Wallis test was used for 3 group comparisons of non-normally distributed quantitative variables while post hoc analysis was performed using Mann-Whitney *U* test. Logistic

regression analysis was used to adjust for covariates while testing the effect of the thyroid status on B12 deficiency. A different model was used to determine the effect of TPO antibodies positivity on B12 deficiency after control for other covariates. Data was presented as odds ratio (OR) and 95% confidence interval (CI). A *P*-value <.05 was considered to be statistically significant.

Ethical consideration

This research was approved by the institutional research committee of Jahra Hospital (J1-23122018). All participants provided informed consent. Data confidentiality was assured. No personally identifiable information was collected.

Results

Clinical characteristics and biochemical measures

Our study included a total of 133 patients divided into 3 groups: 45 patients with OH, 48 patients with SCH, and 40 patients in the control group. The mean age of participants was 34.3 ± 14.4 years and 82% of them were females. The greater number of patients were middle-aged (78%), while adolescents represented 15% of patients and about 7% of patients were 60 years or older. The clinical characteristics, comorbidities, and biochemical measures of participants are shown in Table 1. Details of the euthyroid control group are illustrated in the Supplemental Table S1. None of the patients with comorbidities was using drugs that can cause vitamin B12 deficiency. The frequency of symptoms and signs associated with B12 deficiency is demonstrated in Table 1. None of the participants reported symptoms of forgetfulness, mood impairment, or sore tongue. Symptoms associated with PN were reported more frequently in the hypothyroid groups (OH and SCH) compared to the control group (33.3%, 18.8%, and 5%, respectively; *P* = .004). However, physical signs of peripheral neuropathy were noted in only 9 patients in the whole study population, with no significant difference between the 3 groups (*P* = .067). Other physical examination findings for example, pallor and glossitis were not reported in our cohort. As demonstrated in Table 1, the mean Hb level was 129 ± 18 g/L, the mean MCV was 82 ± 7 fL, and anemia was present in 26.3% of patients with no significant difference across groups (*P* = .963). Most anemic patients (83%) had iron deficiency anemia. There was a statistically significant difference between the groups regarding the TSH and free T4 levels (*P* < .001 and .026, respectively) as shown in Table 1.

Characteristics of the hypothyroid patients

The mean duration of illness in the OH and SCH groups was 6.9 ± 5.57 and 1.48 ± 2.86 years, respectively. In the OH group, 93% of patients were receiving levothyroxine treatment and the mean dose was 97.6 ± 36.6 µg, while in the SCH group, 29% of patients were getting levothyroxine replacement and the mean dose was 50 ± 24 µg. Among the patients who were not receiving levothyroxine replacement in the SCH group, 9 patients were not compliant to treatment and 25 patients were not started on treatment because their TSH levels remained between 4 and 10 mIU/L and they were asymptomatic.[24] Thyroid antibodies (TPO antibodies) were checked in 79/93 of the hypothyroid patients and were positive in 48 (60.8%) of them (25/34 [73.5%] and 23/45 [51.1%] in the OH and SCH groups, respectively).

Assessment of vitamin B12 deficiency

As shown in Table 2, vitamin B12 deficiency was detected in 53 patients with no statistically significant difference between the OH, SCH, and control groups (33.3%, 47.9%, and 37.5%, respectively; *P* = .334). Among the 80 non-deficient patients (B12 level ⩾133 pmol/L), borderline B12 level was identified in 52.5% of them and was significantly more prevalent in the hypothyroid groups (OH and SCH) compared to the control group (*P* = .018), as demonstrated in Table 2. Borderline-to-low B12 levels (defined as serum level ⩽221 pmol/L) were identified in 71.4% of participants and was significantly more prevalent in the hypothyroid groups (OH and SCH) compared to the control group (68.9%, 85.4%, and 57.5%, respectively; *P* = .014) as demonstrated in Table 2.

The distribution of the anemic patients across the spectrum of B12 levels was 17 patients (32.1%), 11 patients (26.2%), and 7 patients (18.4%) in the deficient, borderline, and normal B12 groups, respectively and there was no significant difference among the groups (*P* = .345). None of the patients with B12 deficiency had macrocytosis (MCV > 100 fL) and 39.6% of them had additional iron deficiency.

Among patients with vitamin B12 deficiency, IFAB was detected in 4/53 patients (7.5%); 2/15 patients (13.3%) in the OH group, and 2/23 patients (8.7%) in the SCH group. Also, tTG antibodies were found in 3/53 patients (5.7%); 2/15 patients (13.3%) in the OH group, and 1/15 (6.7%) in the control group. None of them had concomitant IFAB and tTG antibodies.

Logistic regression analysis showed that the effect of the thyroid status on vitamin B12 deficiency remained insignificant (*P* = .172) after adjusting for age (OR, 1; 95% CI, 0.97-1.03; *P* = .777), sex (OR, 1.01; 95% CI, 0.28-3.60; *P* = .985), and the duration of illness (OR, 0.97; 95% CI, 0.87-1.08; *P* = .612). Another model was used to determine the effect of TPO antibodies positivity on B12 deficiency and was found to be statistically insignificant (OR, 0.69; 95% CI, 0.3-1.57; *P* = .147) after control for other variables that is, age, sex, thyroid status, and duration of illness.

Table 1. Clinical and laboratory data of all participants.

	OH	SCH	CONTROL	TOTAL	*P* VALUE
	N = 45 (33.8%)	N = 48 (36.1%)	N = 40 (30.1%)	N = 133	
Age (y), mean ± SD	40.6 ± 13.9	30.7 ± 13.7	31.7 ± 13.7	34.3 ± 14.4	.243
Age groups, n (%)					
12-19 y	2 (4.4)	11 (22.9)	7 (17.5)	20 (15)	
20-39 y	17 (37.8)	27 (56.2)	22 (55)	66 (49.6)	
40-59 y	21 (46.7)	8 (16.7)	9 (22.5)	38 (28.6)	
⩾60 y	5 (11.1)	2 (4.2)	2 (5)	9 (6.8)	
Sex, n (%)					.165
Male	7 (15.6)	6 (12.5)	11 (27.5)	24 (18)	
Female	38 (84.4)	42 (87.5)	29 (72.5)	109 (82)	
Comorbidities, n (%)					
Diabetes	3 (6.7)	0 (0)	0 (0)	3 (2.3)	
Hypertension	3 (6.7)	0 (0)	6 (15)	9 (6.8)	
Others[a]	1 (2.2)	2 (4.2)	7 (17.5)	10 (7.5)	
All comorbidities	7 (15.6)	2 (4.2)	13 (32.5)	22 (16.5)	
Symptoms associated with PN[b], n (%)	15 (33.3)	9 (18.8)	2 (5)	26 (19.5)	.004
Examination findings associated with PN[c], n (%)	3 (6.7)	6 (12.5)	0 (0)	9 (6.8)	.067
Hemoglobin *(NR*, 120-150 g/L*)*, mean ± SD	127 ± 17	128 ± 18	131 ± 18	129 ± 18	.519
MCV *(NR*, 83-101 fL*)*, mean ± SD	83 ± 7	81 ± 7	82 ± 7	82 ± 7	.389
Anemia[d], n (%)	12 (26.7)	12 (25)	11 (27.5)	35 (26.3)	.963
TSH *(NR, 0.3-4 mIU/L)*, median (IQR)	3.1 (1.95-7)	5.4 (3.54-7.15)	2 (1.33-2.53)	3.1 (1.85-5.58)	<.001[e]
Free T4 *(NR*, 7.5-21.1 pmol/L*)*, mean ± SD	12.2 ± 3.32	10.9 ± 1.97	11.5 ± 1.4	11.5 ± 2.44	.026
Patients receiving levothyroxine replacement, n (%)	42 (93.3)	14 (29.2)	NA		

Abbreviations: IQR, interquartile range; MCV, mean corpuscular volume; n, number; NA, not applicable; NR, normal range; OH, overt hypothyroidism; PN, peripheral neuropathy; SCH, subclinical hypothyroidism; SD, standard deviation; TSH, thyroid-stimulating hormone; T4, thyroxine.
[a]Other comorbidity included: renal, respiratory, neurological, and autoimmune diseases.
[b]Numbness, pain, or paresthesia in the lower limbs.
[c]Peripheral sensory deficits, diminished deep tendon reflexes, and muscle weakness.
[d]Number of anemic female patients in OH, SCH, and control groups was (12, 11, 9, respectively).
[e]Post hoc analysis revealed a significant difference between the control group and both the OH group ($P = .005$) and the SCH group ($P < .001$).

Discussion

Despite a lot of research, there continues to be a debate about the association of hypothyroidism with B12 deficiency. Although some previous studies have demonstrated an association between hypothyroidism and vitamin B12 deficiency,[12,13] this cannot be considered conclusive because other studies did not find an association between them.[11,17,18]

In our study, the mean age of our patients was 34.3 ± 14.4 years. The aging of the population results in an increasing prevalence of both hypothyroidism and cobalamin deficiency.[3,8] In our study, 7% of patients were 60 years or older, and thence aging is not considered as a contributing factor for B12 deficiency in our cohort. Females represented the majority of the hypothyroid group, which is consistent with the known high prevalence of hypothyroidism among females.[25,26]

The median serum TSH levels were higher in both the OH and the SCH groups compared to the control group. In the OH group, this may reflect undertreatment or noncompliance

Table 2. Prevalence of low and borderline vitamin B12 levels in the study groups.

	OH	SCH	CONTROL	TOTAL	*P* VALUE
Vitamin B12 deficiency, n (%)					.334
<133 pmol/L	15 (33.3)	23 (47.9)	15 (37.5)	53 (39.8)[a]	
⩾133 pmol/L	30 (66.7)	25 (52.1)	25 (62.5)	80 (60.2)	
Borderline vitamin B12, n (%)					.018
133-221 pmol/L	16 (53.3)	18 (72)	8 (32)	42 (52.5)	
>221 pmol/L	14 (46.7)	7 (28)	17 (68)	38 (47.5)	
Borderline-to-low vitamin B12, n (%)					.014
⩽221 pmol/L	31 (68.9)	41 (85.4)	23 (57.5)	95 (71.4)	
>221 pmol/L	14 (31.1)	7 (14.6)	17 (42.5)	38 (28.6)	

Abbreviations: n, number; OH, overt hypothyroidism; SCH, subclinical hypothyroidism.
[a]All B12 deficient patients were prescribed B12 supplementation and scheduled for repeat testing. Only 23/53 patients came for follow-up and most of them (82.6%) achieved B12 levels ⩾133 pmol/l after treatment.

with replacement. In the SCH group, this may be related to the lower number of patients receiving replacement therapy. About half of the patients in the SCH group had positive TPO antibodies. In accordance with our results, the reported rate of TPO antibodies positivity in SCH ranged from 30% to 67%.[27] In patients with OH due to Hashimoto's thyroiditis, TPO antibodies are positive in almost 100% of them.[28] Our results revealed that 73.5% of OH patients had positive TPO antibodies. This finding can be explained by the prolonged illness (6.9 ± 5.57 years) and the considerable number of patients on levothyroxine replacement in the group (93%) as levels of TPO antibodies decline gradually over time and decrease with levothyroxine treatment.[28,29] In line with our results, other studies reported thyroid antibodies positivity in 58% to 67% of hypothyroid patients.[11,13]

Numbness and paresthesia of the lower limbs was reported with a higher frequency among OH and SCH patients compared to controls. However, we found a lower number of patients with confirmed findings on physical examination with no difference among the groups. Similarly, Jabbar et al[13] found that 55% to 60% of B12-deficient hypothyroid patients complain of numbness and paresthesia, but none of them had impaired vibration or position sense on physical examination and only 26% of them had impaired reflexes. Although paresthesia, numbness, and impaired reflexes are common findings in vitamin B12 deficiency, this difference cannot be solely attributed to vitamin B12 deficiency because peripheral neuropathy symptoms and signs are common in hypothyroid patients (29% and 42%, respectively).[30] Furthermore, the clinical features of cobalamin deficiency are subtle and can be missed if not evaluated by the measurement of serum B12 levels. In hypothyroid patients, surrogate clinical findings cannot be merely relied upon to diagnose patients with cobalamin deficiency.[31]

Although serum B12 is the primary test in clinical practice, it has poor sensitivity and specificity for the reliable recognition of B12 deficiency. To overcome these problems, other tests can be used for example, plasma methylmalonic acid (MMA), homocysteine, and serum holotranscobalamin. However, these tests are expensive, not routinely available, do not have clearly defined cut-points to denote deficiency, and their role in the clinical practice is still unclear.[32]

In our study, we used a serum B12 level of <133 pmol/L to detect deficiency according to the manufacturer's cut-off points. We preferred the manufacturer's cut-points because of the great variability between laboratories and in the literature in reporting B12 results.[32] However, the cut-off point to define a borderline B12 status or SCCD is less clear, and we chose a serum B12 level of 133 to 221 to define SCCD in our study.[3,21] However, the performance of serum B12 levels in the diagnosis of SCCD is less reliable and there is a greater cut-points variability compared to clinical B12 deficiency.[33]

Our results suggested that there is an insignificant difference between primary hypothyroidism and controls as regards the prevalence of B12 deficiency. We reported a prevalence of 33.3% in OH, 47.9% in SCH, and 37.5% in the control group. This is higher than the prevalence reported before among Kuwaiti population (1.3%-5.6%), although this study reported the prevalence of B12 deficiency anemia among a specific group of iron sufficient population and included all age groups (neonates, pediatric, and adults).[34] Larger scale studies are needed to confirm this finding and actions to be considered accordingly.

Previous studies investigating the prevalence of B12 deficiency in hypothyroidism have demonstrated varying results. Our results are broadly in line with other studies such as Lippi et al who could not find a substantial difference for serum B12 across the range of different TSH levels. The authors did not

recommend routine screening for B12 deficiency in hypothyroid patients. However, the assay used (Chiron Diagnostics) and the cut-point used to define B12 deficiency (<125 pmol/L) were different from our study.[18] Das et al found that 10% of hypothyroid patients (OH and SCH) have B12 deficiency anemia and that rate was like the general population estimates. However, this study lacks a control group and the cut-point used to define B12 deficiency is unknown.[11] Another study of B12 deficiency among anemic hypothyroid patients, measured by Beckman Coulter Synchron LX20 and using a cut-point of <140 pmol/L, found that 25.6% of SCH patients and 18.6% of OH patients had B12 deficiency compared to 19.2% of controls, and the difference was not significant ($P = .691$).[17] A recent study found that there was no significant difference in serum B12 levels among euthyroid, hyperthyroid, and hypothyroid patients ($P = .33$).[35]

Other studies have reported a higher prevalence of B12 deficiency among hypothyroid patients such as Jabbar et al[13] who found 40.5% of hypothyroid patients to have cobalamin deficiency, and Aktaş[16] who reported that 46% of hypothyroid patients have B12 deficiency. Both studies used a cut-point of <148 pmol/L and recommended screening hypothyroid patients for B12 deficiency. Another study by Jaya Kumari et al[31] reported a B12 deficiency prevalence rate of 44.5% among patients with AITD which is close to our results using the same cut-point to define B12 deficiency (<133 pmol/L). Although the estimated prevalence of B12 deficiency in these studies is numerically close to our findings, we argue against the significance of these results due to the lack of comparison with a control group. These results may reflect the populations' nutritional status.

The variable prevalence rates of B12 deficiency among hypothyroid patients reported in the previous studies, ranging from 10% to 46%, maybe due to the difference in the nutritional status and the different laboratory cut points used to define B12 deficiency. Furthermore, the possibility that cut points used to define cobalamin deficiency in euthyroid patients may not apply to OH and SCH cannot be excluded.

Although some studies supported the routine screening of hypothyroid patients for B12 deficiency,[12,13,16] we do not recommend the routine screening of hypothyroid patients for B12 deficiency. This is in line with the recommendation of the British Committee for Standards in Hematology (BCSH) that do not support routine B12 screening for non-specific symptoms without objective parameters and requires repeat testing to confirm the persistence of low B12 levels and confirmation with IFAB or other second-line tests such as MMA, homocysteine, and holotranscobalamin before treatment.[32] Also, screening may be needed in cases of polyglandular autoimmune syndromes due to the association with autoimmune gastritis (PA) and celiac disease, both can cause cobalamin deficiency.[36]

Anemia prevalence was the same among the study groups. Previous studies have reported variable rates of prevalence of anemia among hypothyroid patients. One study found that 39% and 43% of SCH and OH patients, respectively, have anemia and that rate was higher compared to 26% of controls ($P = .002$).[17] Another study found that 16.3% of patients with autoimmune thyroid disease (AITD) have anemia.[37] A higher rate of anemia was reported in a previous study that included OH and SCH patients (26.6% and 73.2%, respectively).[11] Despite the variability of prevalence rates, all the previously mentioned studies demonstrated that the most common cause of anemia in hypothyroid patients is anemia of chronic disease. The anemia of hypothyroidism has a multifactorial etiology linked to the suppressed bone marrow and erythropoietin production. Furthermore, concurrent autoimmune diseases such as PA and celiac disease leading to hematinic deficiency (eg, iron, vitamin B12, and folate) contribute to the pathogenesis of anemia in hypothyroidism.[38]

We could not find a significant difference in anemic patients' distribution across the spectrum of B12 levels. A similar pattern of results was obtained in previous studies. Mehmet et al[17] reported no difference in the rates of anemia arising from vitamin B12 deficiency between SCH, OH, and controls (25.6%, 18.6%, and 19.2%, respectively; 0.686). Jabbar et al[13] did not find an increased prevalence of anemia in B12-deficient hypothyroid patients compared to the non-deficient hypothyroid group.

We did not find any of our B12-deficient hypothyroid patients having macrocytosis. In line with our findings, a large population-based study found that anemia with thyroid dysfunction is rarely macrocytic.[39] An MCV higher than the reference range is not necessary for cobalamin deficiency. It is common to have B12 deficiency with normal or low MCV due to concurrent iron deficiency or hemoglobinopathies.[40] Moreover, the presence of macrocytic megaloblastic anemia is a feature of severe cobalamin deficiency observed with PA or gut surgeries but rarely seen with mild deficiency due to dietary insufficiency or drug use (eg, metformin).[41] Thus, the absence of macrocytosis among our patients can be explained by the high rate of iron deficiency prevalence.

In our study, 20% of controls have borderline B12 levels or SCCD, which is close to the prevalence reported previously in other studies.[42] Although a substantial number of hypothyroid patients (OH and SCH) had SCCD compared to the control group, the clinical significance of this finding is questionable. SCCD is merely a laboratory finding without a strong impact on the patient. It is usually not associated with the clinical findings of B12 deficiency, lacks the other biochemical markers of cobalamin deficiency for example, elevated MMA and homocysteine levels, and usually does not progress to clinical B12 deficiency with time.[43] Currently, there is no convincing evidence of a clear benefit in treating SCCD. Guidelines do not recommend routine treatment of SCCD. Instead, it is recommended to rely on the clinical judgment, repeat serum B12 levels to confirm the deficiency, and perform some second-line tests for example, MMA and homocysteine if deficiency is highly suspected, before any empirical treatment trials.[32]

PA was detected in 7.5% of our hypothyroid patients by positive IFAB. Das et al[11] reported that 5% of primary hypothyroidism patients have PA detected by parietal cell antibodies (PCA). A higher prevalence (30%) of PCA positivity among patients with AITD was reported in another study.[12] Wang et al[37] found 25% of patients with AITD to have positive PCA. Another study has reported that 20% of patients with AITD have positive PCA and identified the presence of elevated levels of thyroid autoantibodies as a risk factor for PCA positivity.[44] The sensitivity of IFAB and PCA in diagnosing PA is 50% and 80%, respectively, whereas the specificity is 100% and 50% to 100%, respectively. PCA can be positive in normal subjects and a positive PCA is not conclusive for PA.[2] In a large cohort of AITD patients, 30% had positive PCA (13% in the younger patients vs 42% in the geriatric population). Twenty-one percent of them transformed spontaneously to a PCA-negative state during follow-up.[45] Due to the lower specificity, it is not recommended to use PCA to diagnose PA. Instead, all patients with low serum cobalamin levels suspected to have PA should be tested for IFAB according to the BCSH guidelines.[32]

Celiac disease is usually suspected in hypothyroidism when patients have raised daily requirements of levothyroxine or during the evaluation of iron deficiency anemia.[46] However, celiac disease is a less popular cause of B12 deficiency among hypothyroid patients. We found 13.3% of our hypothyroid patients to have positive tTG antibodies compared to 6.7% of controls. In line with our results, tTG antibodies were positive in 15% of Hashimoto's thyroiditis patients compared to 7% of healthy controls in a previous study.[47] Another study reported higher tTG antibodies levels among patients with AITD compared to controls (7% vs 3.5%, $P = .015$).[48] Tissue transglutaminase antibodies are highly specific and sensitive tools for the diagnosis of celiac disease.[49] Ness-Abramof et al[12] recommended screening B12-deficient AITD patients for celiac disease using tTG antibodies if they were normo-gastrinemic. Lastly, we did not find an association between TPO antibodies positivity and cobalamin deficiency. This finding was reported in other studies[31] and implies that factors other than autoimmune diseases for example, nutritional status, decreased intestinal absorption due to slow gut motility, and bacterial overgrowth may play a role in B12 deficiency among hypothyroid patients. Limitations in our study included the small number of patients and the single-center nature of the study. The role of dietary B12 deficiency cannot be excluded as we did not perform a dietary evaluation for our patients. Also, IFAB and tTG antibodies were not measured in the SCCD group which may identify further patients with PA or celiac disease. Finally, we did not evaluate other markers of B12 deficiency for example, MMA, homocysteine, and holotranscobalamin.

Conclusion

Taken together, our results do not support the hypothesis of an increased prevalence of vitamin B12 deficiency among patients with primary hypothyroidism. The reported prevalence probably reflects the nutritional state of the studied population. We believe that routine screening for B12 deficiency among those patients is not recommended unless B12 deficiency is clinically suspected or in the presence of the polyglandular autoimmune syndrome. In hypothyroid patients with B12 deficiency, pernicious anemia and celiac disease may be possible causes of the deficiency. SCCD is more prevalent among OH and SCH patients, but the clinical significance of this finding is questionable. Further studies on a larger population may be needed to investigate the role of other markers of B12 deficiency (eg, MMA, homocysteine, and holotranscobalamin) and evaluate the benefits of B12 replacement on the clinical outcomes in B12 deficiency and SCCD.

Acknowledgements

The authors would like to thank Ms. Sara Mohamed for the significant help in data entry and typing.

Author Contribution(s)

Mohamed Aon: Design, Methodology, Data curation, Data analysis, Writing original draft and Approval of the final version.
Sherif Taha: Methodology, Data curation, Review, Editing and Approval of the final version.
Khaled Mahfouz: Data curation, Review, Editing and Approval of the final version.
Mohamed M Ibrahim: Data curation, Review, Editing and Approval of the final version.
Ahmed H Aoun: Methodology, Data curation, Data analysis, Writing original draft and Approval of the final version.

ORCID iDs

Mohamed Aon https://orcid.org/0000-0001-9109-1697
Mohamed M Ibrahim https://orcid.org/0000-0002-6287-370X
Ahmed H Aoun https://orcid.org/0000-0003-4918-0965

Supplemental Material

Supplemental material for this article is available online.

REFERENCES

1. Watanabe F. Vitamin B12 sources and bioavailability. *Exp Biol Med*. 2007;232:1266-1274.
2. Stabler SP. Vitamin B12 deficiency. *N Engl J Med*. 2013;368:149-160.
3. Allen LH. How common is vitamin B-12 deficiency? *Am J Clin Nutr*. 2009; 89:693S-696S.
4. Rothen JP, Walter PN, Tsakiris DA, Infanti L, Hersberger KE, Arnet I. Identification of patients with cobalamin deficiency crucially depends on the diagnostic strategy. *Clin Lab*. 2021;67:1229-1235. doi:10.7754/Clin.Lab.2020.200912
5. İspir E, Serdar MA, Ozgurtas T, et al. Comparison of four automated serum vitamin B12 assays. *Clin Chem Lab Med*. 2015;53:1205-1213.
6. Kulnigg-Dabsch S. Autoimmune gastritis. *Wien Med Wochenschr*. 2016;166: 424-430.
7. Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. *Lancet*. 2017;390:1550-1562.
8. Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. *J Clin Endocrinol Metab*. 2014;99:923-931.

9. Almandoz JP, Gharib H. Hypothyroidism: etiology, diagnosis, and management. *Med Clin North Am*. 2012;96:203-221.
10. Collins AB, Pawlak R. Prevalence of vitamin B-12 deficiency among patients with thyroid dysfunction. *Asia Pac J Clin Nutr*. 2016;25:221-226.
11. Das C, Sahana PK, Sengupta N, Giri D, Roy M, Mukhopadhyay P. Etiology of anemia in primary hypothyroid subjects in a tertiary care center in Eastern India. *Indian J Endocrinol Metab*. 2012;16:S361-S363.
12. Ness-Abramof R, Nabriski DA, Braverman LE, et al. Prevalence and evaluation of B12 deficiency in patients with autoimmune thyroid disease. *Am J Med Sci*. 2006;332:119-122.
13. Jabbar A, Yawar A, Waseem S, et al. Vitamin B12 deficiency common in primary hypothyroidism. *J Pak Med Assoc*. 2008;58:258-261.
14. What is Celiac Disease? Celiac Disease Foundation. Accessed September 24, 2021. https://celiac.org/about-celiac-disease/what-is-celiac-disease/
15. Dahele A, Ghosh S. Vitamin B12 deficiency in untreated celiac disease. *Am J Gastroenterol*. 2001;96:745-750.
16. Aktaş HŞ. Vitamin B12 and Vitamin D levels in patients with autoimmune hypothyroidism and their correlation with anti-thyroid peroxidase antibodies. *Med Princ Pract*. 2020;29:364-370.
17. Mehmet E, Aybike K, Ganidagli S, Mustafa K. Characteristics of anemia in subclinical and overt hypothyroid patients. *Endocr J*. 2012;59:213-220.
18. Lippi G, Montagnana M, Targher G, Salvagno GL, Guidi GC. Prevalence of folic acid and vitamin B12 deficiencies in patients with thyroid disorders. *Am J Med Sci*. 2008;336:50-52.
19. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. *Am J Clin Nutr*. 2005;82:442-450.
20. OpenEpiMenu. Accessed December 15, 2018. https://www.openepi.com/Menu/OE_Menu.htm
21. Aroda VR, Edelstein SL, Goldberg RB, et al. Long-term metformin use and vitamin B12 deficiency in the Diabetes Prevention Program Outcomes Study. *J Clin Endocrinol Metab*. 2016;101:1754-1761.
22. Cappellini MD, Motta I. Anemia in clinical practice-definition and classification: does hemoglobin change with aging? *Semin Hematol*. 2015;52:261-269.
23. Auerbach M, Adamson JW. How we diagnose and treat iron deficiency anemia: diagnose and treat iron deficiency anemia. *Am J Hematol*. 2016;91:31-38.
24. Pearce SH, Brabant G, Duntas LH, et al. 2013 ETA guideline: management of subclinical hypothyroidism. *Eur Thyroid J*. 2013;2:215-228.
25. Aoki Y, Belin RM, Clickner R, Jeffries R, Phillips L, Mahaffey KR. Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999-2002). *Thyroid*. 2007;17:1211-1223.
26. McLeod DSA, Caturegli P, Cooper DS, Matos PG, Hutfless S. Variation in rates of autoimmune thyroid disease by race/ethnicity in US military personnel. *JAMA*. 2014;311:1563.
27. Collet TH, Bauer DC, Cappola AR, et al. Thyroid antibody status, subclinical hypothyroidism, and the risk of coronary heart disease: an individual participant data analysis. *J Clin Endocrinol Metab*. 2014;99:3353-3362.
28. Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A. Antithyroid peroxidase autoantibodies in thyroid diseases. *J Clin Endocrinol Metab*. 1990;71:661-669.
29. Peeters RP. Subclinical hypothyroidism. *N Engl J Med*. 2017;376:2556-2565.
30. Duyff RF, Van den Bosch J, Laman DM, van Loon BJP, Linssen WHJP. Neuromuscular findings in thyroid dysfunction: a prospective clinical and electrodiagnostic study. *J Neurol Neurosurg Psychiatry*. 2000;68:750-755.
31. Jaya Kumari S, Bantwal G, Devanath A, Aiyyar V, Patil M. Evaluation of serum vitamin B12 levels and its correlation with anti-thyroperoxidase antibody in patients with autoimmune thyroid disorders. *Indian J Clin Biochem*. 2015;30:217-220.
32. Devalia V, Hamilton MS, Molloy AM; British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *Br J Haematol*. 2014;166:496-513.
33. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *Am J Clin Nutr*. 2011;94:348S-358S.
34. Al Zenki S, Alomirah H, Al Hooti S, et al. Prevalence and determinants of anemia and iron deficiency in Kuwait. *Int J Environ Res Public Health*. 2015;12:9036-9045.
35. Atak B, Aktas G, Duman TT. Association between thyroid hormone status and complete blood count parameters in anemic patients. *Malays J Med Health Sci*. 2021;17:101-104.
36. Husebye ES, Anderson MS, Kämpe O. Autoimmune polyendocrine syndromes. *N Engl J Med*. 2018;378:1132-1141.
37. Wang YP, Lin HP, Chen HM, Kuo YS, Lang MJ, Sun A. Hemoglobin, iron, and vitamin B12 deficiencies and high blood homocysteine levels in patients with anti-thyroid autoantibodies. *J Formos Med Assoc*. 2014;113:155-160.
38. Szczepanek-Parulska E, Hernik A, Ruchała M. Anemia in thyroid diseases. *Pol Arch Intern Med*. 2017;127:352-360. doi:10.20452/pamw.3985
39. M'Rabet-Bensalah K, Aubert CE, Coslovsky M, et al. Thyroid dysfunction and anaemia in a large population-based study. *Clin Endocrinol*. 2016;84:627-631.
40. Damon LE, Andreadis CB. Blood disorders. In: Maxine A Papadakis, Stephen J McPhee, eds. *Current Medical Diagnosis and Treatment*. 60th ed. McGraw Hill; 2021:519-520.
41. Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. *Blood*. 2017;129:2603-2611.
42. Bailey RL, Carmel R, Green R, et al. Monitoring of vitamin B-12 nutritional status in the United States by using plasma methylmalonic acid and serum vitamin B-12. *Am J Clin Nutr*. 2011;94:552-561.
43. Carmel R. Diagnosis and management of clinical and subclinical cobalamin deficiencies: why controversies persist in the age of sensitive metabolic testing. *Biochimie*. 2013;95:1047-1055.
44. García García B, Gimeno Orna JA, Aguillo Gutiérrez E, et al. Prevalencia y factores predictivos de la presencia de anticuerpos anticélula parietal gástrica en la enfermedad tiroidea autoinmune. *Endocrinol Nutr*. 2010;57:49-53.
45. Checchi S, Montanaro A, Ciuoli C, et al. Prevalence of parietal cell antibodies in a large cohort of patients with autoimmune thyroiditis. *Thyroid*. 2010;20:1385-1389.
46. Zubarik R, Ganguly E, Nathan M, Vecchio J. Celiac disease detection in hypothyroid patients requiring elevated thyroid supplementation: A prospective cohort study. *Eur J Intern Med*. 2015;26:825-829.
47. Hadizadeh Riseh S, Abbasalizad Farhang M, Mobasseri M, Asghari Jafarabadi M. The relationship between thyroid hormones, antithyroid antibodies, anti-tissue transglutaminase and anti-gliadin antibodies in patients with Hashimoto's thyroiditis. *Acta Endocrinol*. 2017;13:174-179.
48. Marwaha RK, Garg MK, Tandon N, et al. Glutamic acid decarboxylase (anti-GAD) & tissue transglutaminase (anti-TTG) antibodies in patients with thyroid autoimmunity. *Indian J Med Res*. 2013;137:82-86.
49. Hopper AD, Hadjivassiliou M, Hurlstone DP, et al. What is the role of serologic testing in celiac disease? A prospective, biopsy-confirmed study with economic analysis. *Clin Gastroenterol Hepatol*. 2008;6:314-320.