Prevalence of etiological factors for vitamin B 12 deficiency in a population of Palmira, Valle, Colombia. Retrospective cross-sectional study

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Abstract

Introduction: Vitamin B 12 deficiency is a clinical condition with multiple causes. Its main clinical manifestations are hematologic and neuropsychiatric.

Objectives: to establish the sociodemographic characteristics and the prevalence of etiological factors for vitamin B 12 deficiency in a population of Palmira, Colombia.

Materials and methods: A retrospective cross-sectional study was developed in a tertiary referral center in Palmira, Colombia. The study period was from March 1st, 2020, to March 1st, 2023. Electronic medical records were reviewed to obtain information on patients' clinical characteristics, comorbidities, and laboratory tests.

Results: Vitamin B 12 deficiency mainly affects the adult population and the elderly. Among the etiological factors, autoimmune diseases (mainly primary hypothyroidism due to Hashimoto's thyroiditis) and the consumption of some medications such as metformin and proton pump inhibitors stood out with greater prevalence.

Conclusions: vitamin B12 deficiency is sought in the medical routine, it affects the elderly population and autoimmune thyroid disease, and metformin consumption are the main etiological factors.

Keywords: vitamin B12, anemia, proton pump inhibitors, metformin, hypothyroidism.

Resumen

Introducción: la deficiencia de vitamina B 12 es una condición clínica de múltiples causas. Sus principales manifestaciones clínicas son hematológicas y neuropsiquiátricas.

Objetivo: establecer las características sociodemográficas y la prevalencia de factores etiológicos para deficiencia de vitamina B 12 en una población de Palmira, Colombia.

Materiales y métodos: se desarrolló un estudio retrospectivo de corte transversal en un centro de referencia terciario de Palmira, Colombia. El período de estudio fue de 01 marzo de 2020 a 01 marzo de 2023. Se revisaron los registros médicos electrónicos para obtener la información sobre las características clínicas de los pacientes, comorbilidades y pruebas de laboratorio.

Resultados: la deficiencia de vitamina B 12 afecta principalmente a la población adulta y a los ancianos. Dentro de los factores etiológicos, se destacó con mayor prevalencia las enfermedades autoinmunes (principalmente el hipotiroidismo primario por tiroiditis de Hashimoto) y el consumo de algunos medicamentos como metformina e inhibidores de bombas de protones.

Conclusiones: la deficiencia de la vitamina B12 es buscada en la rutina médica, afecta a la población mayor y la enfermedad tiroidea autoinmune y el consumo de metformina son los principales factores etiológicos.

Palabras clave: vitamina B 12, anemia, inhibidores de bombas de protones, metformina, hipotiroidismo

Introduction

Vitamin B 12 deficiency presents a wide variety of clinical manifestations and a large repertoire of causes (1-3). Its incidence increases with aging (4) and its main clinical manifestations are neuropsychiatric and hematological (4). This vitamin is necessary for DNA synthesis in hematopoietic precursors. Hematological findings include macrocytosis, thrombocytopenia and hypersegmented neutrophils. In some cases, it can be manifested with pancytopenia and hemolysis due to ineffective hematopoiesis (5). Less common are neurological symptoms that may include paresthesias due to peripheral neuropathy, which in its most severe form causes axonal degeneration in the peripheral nerves and in the lateral and posterior columns of the spinal cord, triggering a clinical condition known as subacute combined degeneration (6). Likewise, neuropsychiatric manifestations including personality changes and psychosis may occur. Finally, vitamin B 12 deficiency is recognized as a cause of dementia (6).

The main causes of deficiency are related to low intake due to malnutrition, vegan/vegetarian diets (since vitamin B 12 is of animal origin) and due to gastrointestinal malabsorption disorders related to medications, autoimmune atrophic gastritis, achlorhydria, ileal damage and bariatric surgery, among others (7). Autoimmune atrophic chronic gastritis (or pernicious anemia) is distinguished by the presence of autoantibodies against intrinsic factors and gastric parietal cells, which play a fundamental role in the gastrointestinal absorption pathway of vitamin B12. The association of pernicious anemia with other autoimmune diseases, such as Hashimoto's thyroiditis, type 1 diabetes mellitus and vitiligo, has been described (6). It seems clear that autoimmune thyroid disease is related to autoimmune atrophic chronic gastritis, so it has been common to find vitamin B12 deficiency in subjects with autoimmune thyroid disease (8). The consumption of drugs that interfere with intestinal absorption is widely described, with metformin and proton pump inhibitors being the most relevantly mentioned (9).

For laboratory diagnosis, limitations have been reported to support the diagnosis, as the rate of false positives and false negatives is worrisome (2).

Some authors have recommended measurement of methylmalonic acid, total homocysteine or both to confirm the diagnosis of vitamin B12 deficiency (1), especially when there is marginal deficiency (serum levels between 148 to 221 pmol/L) (4). In addition, patients with pernicious anemia with antibodies to intrinsic factors may show falsely elevated levels (10). It has been suggested that the appropriate laboratory assay would be one that measures the levels of active vitamin b12, i.e., the blood fraction bound to transcobalamin (holoTC) (3).

Materials and methods

A retrospective cross-sectional study was carried out in a tertiary referral center in Palmira, Colombia. The aim of the study was to perform sociodemographic characterization and to establish the prevalence of the different etiologies described in the literature for vitamin B 12 deficiency. The study period was from March 1st, 2020 to March 1st, 2023. The institution's clinical laboratory provided the results of vitamin B 12 (cyanocobalamin) levels performed during the study period. Patients with cyanocobalamin levels ≤200 pg/mL (picograms per milliliter) were included. Electronic medical records were reviewed to obtain sociodemographic information and to establish the different known etiologic factors for vitamin B 12 deficiency. Data was collected on patients' clinical characteristics, comorbidities, and laboratory tests. The prevalence of different etiological factors for vitamin B12 deficiency reported in the medical literature was sought to establish, such as: strict vegetarian or vegan diet, alcoholism, liver cirrhosis, malnutrition, primary hypothyroidism, primary hyperthyroidism, vitiligo, gastritis, especially atrophic gastritis (autoimmune) and Helicobacter pylori infection; inflammatory bowel disease, history of bariatric surgery, history of surgery with stomach or ileum resection; autoimmune diseases (such as Sjögren's syndrome, systemic lupus erythematosus -SLE- and rheumatoid arthritis); medications (proton pump inhibitors, metformin), type 1 and type 2 diabetes mellitus and chronic infections (such as HIV, hepatitis B, hepatitis C, syphilis) were searched. The prevalence of concomitant iron and folic acid deficiency was also sought to be established. Finally, it was explored for signs/symptoms including glossitis, chronic diarrhea, peripheral neuropathy, dementia, and hemolysis associated with vitamin B12 deficiency. Administrative and bioethical permission was obtained from the study center (High Complexity Clinic Santa Bárbara Palmira, Colombia).
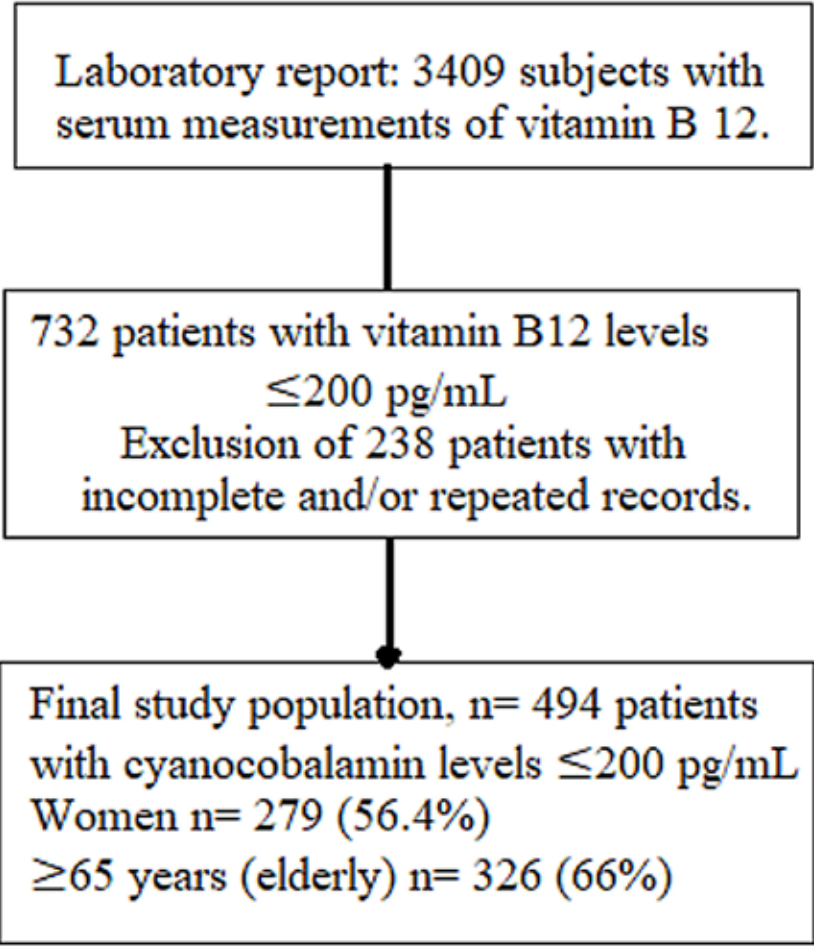
Statistical analysis

Descriptive statistics were calculated. Normality was assessed using the Kolmogorov Smirnov test or the Shapiro Wilk test when appropriate. For continuous variables with assumptions of normality, Student's t-tests were performed. Nonparametric Wilcoxon or Man Whitney U tests were used for continuous variables that did not meet normality assumptions. Continuous variables were presented as mean ± standard deviation or as medians and interquartile ranges (IQR) when appropriate. Categorical variables were presented as frequency and percentage. Categorical variables were analyzed using the Chi-square test or Fisher's exact test when appropriate. Free public domain Epi Info™ software was used for all statistical analyses.

Results

A population of 494 patients was analyzed. Fifty-six percent of the population was female. The majority of the population were adults or elderly. The median age was 63 years with an interquartile range (IQR) of 62-81years. Seventy-five percent of the population was ≥62 years old. The patient selection diagram is presented in Figure 1. Table 1 summarizes the main clinical, demographic and laboratory characteristics.

Fig

Laboratory report: 3409 subjects with serum measurements of vitamin B 12.

732 patients with vitamin B12 levels ≤200 pg/mL
Exclusion of 238 patients with incomplete and/or repeated records.

Final study population, n= 494 patients with cyanocobalamin levels ≤200 pg/mL
Women n= 279 (56.4%)
≥65 years (elderly) n= 326 (66%)

Table 1. Main clinical, demographic and laboratory characteristics

Variable	Total, n=494(%)	Men, n=215(%)	Women, n=279(%)	P value	≥65years old n=326(%)	<65years old n=168(%)	P value
Age, years, median (IQR)	74 (59-82)	75 (63-83)	72(52-82)	0.01	79(74-85)	50 (40-59)	0.001
Vitamin B 12 levels, median (IQR)	166 (135-185)	167 (134-185)	165 (135-184)	0.9	168 (134-184)	162(140-186)	0.6
Alcoholism	20/473 (4.2)	14/206(6.8)	6/267(2.2)	0.01	11/319(3.4)	9/154(5.8)	0.2
Malnourishment	11/478 (2.3)	7/2010 (3.3)	4/268 (1.5)	0.2	5/321 (1.6)	6/157(3.8)	0.1
Gastritis history	29/480 (6)	8/209 (3.8)	21/271 (7.7)	0.07	16/322 (5)	13/158 (8)	0.1
Bariatric surgery	16/475 (3.4)	2/208 (1)	14/267 (5.2)	0.01	0/320 (0)	16/155(10)	0.001
Any autoimmune disease	194/483(40)	83/211 (39)	111/272 (40)	0.7	141/323 (43)	53/160 (33)	0.02
Primary hypothyroidism	163/480(34)	78/209 (37)	85/271(31)	0.1	128/321(40)	35/159(22)	0.001
Rheumatoid arthritis	22/476(4.6)	1/206 (0.5)	21/270 (7.8)	0.001	7/318 (2.2)	15/158 (9.5)	0.001
Type 2 diabetes	117/476 (24.6)	52/206 (25)	65/270 (24)	0.8	85/317 (26)	32/159 (20)	0.1
Metformin	82/476 (17)	37/204 (18)	45/272 (16)	0.6	60/319 (18)	22/157 (14)	0.1
Proton pump inhibitor	36/449 (8)	20/197 (10)	16/252 (6.3)	0.1	21/293 (7.2)	15/156 (9.6)	0.3
Hemogram findings							
Hemoglobin, gr/dL, median (IQR)	12.5(11.5-13.7)	12.9 (11.8-14.5)	12.2 (12.2-13.1)	0.001	12.7(11.7-13.8)	12.2(10.8-13.7)	0.030
MCV fL, median (IQR)	90 (86-95)	92 (87-95)	89 (85-94)	0.01	92(87-95)	89 (85-93)	0.007
Erythrocyte distribution width, %, median (IQR)	13.9(13.1-14.9)	13.7(13.7-14.8)	14(13.1-15.1)	0.2	13.8(13.2-14.7)	13.9(13-15.5)	0.4
Platelets, 109/L, median (IQR)	265 (213-327)	244(195-301)	286(227-342)	0.001	250(204-311)	290(230-354)	0.001

Vegetarian or vegan diet was not very prevalent (0.4%), malnutrition was reported in 2.3% of patients and alcoholism had a low prevalence (4.2%), being more common in men than in women (p=0.015, OR 3.1, CI95%1.1 to 8.4); liver cirrhosis was reported in 1%. In gastrointestinal history, the history of gastritis had a prevalence of 6% (without specifying whether it was atrophic gastritis or not). Gastric mucosal atrophy (by endoscopy) was re-

ported in 1.5%. Helicobacter pylori infection was reported in 0.8%. Proton pump inhibitor use was 8%. The prevalence of inflammatory bowel disease was 0.4%. Only 3.4% of patients had a history of bariatric surgery. Glossitis was not reported in any patient at physical examination. Only 0.8% reported chronic diarrhea as a symptom of intestinal malabsorption.

A high prevalence of autoimmune diseases was identified, mainly primary hypothyroidism -Hashimoto's thyroiditis- (34%) and rheumatoid arthritis (4.6%), but a low prevalence of other autoimmune diseases (Graves' disease 1.7%, vitiligo 0.8%, Sjögren's syndrome 0.2%, systemic lupus erythematosus 0.6%, type 1 diabetes mellitus 0.2%). Chronic infections such as syphilis, HIV, hepatitis B and hepatitis C had a low prevalence (0.8%).

The consumption of drugs associated with vitamin B 12 deficiency had a high prevalence, the most consumed being Metformin (17.2%) and then in second place proton pump inhibitors (8%). With respect to metformin the median dose was 850 mg (RIQ 850-2000 mg).

Regarding laboratory variables, hemoglobin levels were close to normal. The prevalence of anemia was 45.7%, most anemias were mild, with a median hemoglobin of 12.5 gr/dL (RIQ 11.5-13.7). Hemoglobin levels were slightly higher in men (12.9 gr/dL vs 12.2 gr/dL, p=0.001) and in the elderly (12.7 gr/dL vs 12.2 gr/DL, p=0.03). The MCV (mean corpuscular volume) value had a median of 90 fL (RIQ 86-95). Macrocytosis was present in 16.2% of the patients evaluated and most of the hemograms showed normocytosis (74%). The MCV of men was slightly higher than that of women (median 92 fL vs 89 fL, p=0.015). Microcytosis had a prevalence of 9.6% and was more common in the non-elderly population (prevalence of 14 % in population <65 years vs 6.9% in population ≥65 years, p=0.02, OR 2.1, 95%CI 1.07 to 4.4) with no differences between gender (p=0.2). Most patients showed normal leukocyte, neutrophil, lymphocyte and platelet counts. Platelet count was slightly lower in men than in women (244 x 109/L vs. 286 x 109/L (p=0.001). Neutropenia and lymphopenia occurred in 19% and 23% of subjects, respectively. Definite thrombopenia had a prevalence of 8%, was more common in men than in women (12.4% vs 4.8%, p= 0.01, OR 2.7, 95%CI 1.2 to 6.2). In none of the patients was methylmalonic acid or homocysteine measured.

Folic acid deficiency was evaluated in 284 patients and deficiency was evidenced in 1.4%. Iron deficiency was evaluated in 238 patients and was confirmed in 24.4%, being more common in women than in men (33% vs.

12.6%, p=0.001 OR 3.4, 95%CI 1.7-6.8). Finally, 1.4% of patients had he-molysis associated with vitamin B 12 deficiency.

Dementia as a manifestation of vitamin B 12 deficiency had a prevalence of 5.3%; peripheral neuropathy associated with vitamin B 12 deficiency had a prevalence of 2.8%, with no differences between genders, but a higher prevalence was observed in the non-elderly population (prevalence of 5.1% in population <65 years vs 1.6% in population ≥65 years, p=0.03, OR 3.3, IC95% 1.07 to 10).

Discussion

In the present study the prevalence of etiological factors for vitamin B 12 deficiency was established. Autoimmune thyroid disease had the highest prevalence with a prevalence of 34%. The causal relationship of primary hypothyroidism due to Hashimoto's disease with vitamin B12 deficiency has been established in the past, the explanation has to do with the existence of antibodies against gastric parietal cells, which are present in the serum of up to 31% of patients with Hashimoto's thyroiditis and up to 24% of patients with Graves' disease (11). The presence of these autoantibodies causes some degree of hypochlorhydria (11), and therefore eventually affects vitamin B12 absorption.

The consumption of some drugs such as metformin and proton pump inhibitors were etiological factors of relevance in this study. Metformin interferes with cubam receptor-mediated absorption in the terminal ileum (12). Since metformin is a cost-effective therapy in diabetes and with cardiovascular benefit, it is advisable to perform periodic monitoring of vitamin B12 in all patients who use it, particularly in those with more than 5 years of treatment (12). Regarding the use of proton pump inhibitors, these are drugs widely used in the general population, they decrease acid secretion leading to vitamin B12 deficiency due to malabsorption, especially when taken for prolonged periods of time (>12 months) (13).

Bariatric surgery had a prevalence of 3.4% as an etiological factor of vitamin B12 deficiency. Bariatric surgery is an effective option for the treatment of obesity. It was present as an etiological factor in 10% of non-elderly patients (<65 years) and was more prevalent in women compared to men (5.2%, vs 1%, p=0.01). Laparoscopic adjustable gastric banding and sleeve gastrectomy have been reported to affect the absorption of iron, selenium and vitamin B12(14). But there are procedures that have a more profound effect on the absorption of vitamins, minerals and essential trace elements

such as Roux-en-Y gastric bypass, jejunoileal bypass and biliopancreatic diversion. Therefore, post-surgical monitoring and individualized nutritional supplementation is essential (14).

Vitamin B12 deficiency is a secondary cause of pancytopenia and can often be mistaken by clinicians for myelodysplastic syndrome (10), in our study, 2.26% of patients presented with pancytopenia. Most of the hemograms showed erythrocyte normocytosis rather than the traditional macrocytosis characteristic of vitamin B12 deficiency. This is not unusual; it has been shown that a certain number of patients, approximately 10%, with erythrocyte normocytosis (with or without anemia), have vitamin B12 deficiency (15).

Limitations

This was a retrospective study whose data acquisition was based on the review of medical records, which implies a high level of underreporting of important etiological factors; therefore, for a more reliable characterization of the information, prospective studies with a more exhaustive evaluation of the etiology of vitamin B12 deficiency are required.

Conclusions

Based on the results presented, it seems clear that vitamin B12 deficiency is frequently sought in the medical routine in a population without anemia and macrocytosis.

The prevalence of hematologic and neurologic involvement was not prominent in this population. It is clear that the presence of normocytosis does not exclude the possibility of vitamin B12 deficiency even in the absence of anemia, and in up to 9.6% of microcytic anemias typically caused by iron deficiency, vitamin B12 deficiency may be present. These findings are in line with those of other authors (15). Therefore, a detailed evaluation of the risk factors of intestinal malabsorption, drug intake, and autoimmune diseases should guide the diagnostic evaluation for vitamin B12 deficiency. The main etiologic factors in our study were autoimmune thyroid disease and the use of drugs such as metformin and proton pump inhibitors.

Conflicts of interest: none.

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Referencies

1. Stabler SP. Clinical practice. Vitamin B12 deficiency. N Engl J Med. 2013;368(2):149-60.

2. Green R, Allen LH, Bjorke-Monsen AL, Brito A, Gueant JL, Miller JW, et al. Vitamin B(12) deficiency. Nat Rev Dis Primers. 2017;3:17040.

3. Green R. Vitamin B(12) deficiency from the perspective of a practicing hematologist. Blood. 2017;129(19):2603-11.

4. Silverstein WK, Cheung MC, Lin Y. Vitamin B(12) deficiency. CMAJ. 2022;194(24):E843.

5. Candelario N, Klein C. Megaloblastic anemia due to severe vitamin B(12) deficiency. Cleve Clin J Med. 2022;89(1):8-9.

6. Htut TW, Thein KZ, Oo TH. Pernicious anemia: Pathophysiology and diagnostic difficulties. J Evid Based Med. 2021;14(2):161-9.

7. Rashid S, Meier V, Patrick H. Review of Vitamin B12 deficiency in pregnancy: a diagnosis not to miss as veganism and vegetarianism become more prevalent. Eur J Haematol. 2021;106(4):450-5.

8. Collins AB, Pawlak R. Prevalence of vitamin B-12 deficiency among patients with thyroid dysfunction. Asia Pac J Clin Nutr. 2016;25(2):221-6.

9. Linder L, Tamboue C, Clements JN. Drug-Induced Vitamin B(12) Deficiency: A Focus on Proton Pump Inhibitors and Histamine-2 Antagonists. J Pharm Pract. 2017;30(6):639-42.

10. Kesbeh Y, Pakbaz Z. Pernicious anemia: a myelodysplastic syndrome look-alike. J Community Hosp Intern Med Perspect. 2019;9(3):240-3.

11. Irvine WJ, Davies SH, Teitelbaum S, Delamore IW, Williams AW. The clinical and pathological significance of gastric parietal cell antibody. Ann N Y Acad Sci. 1965;124(2):657-91.

12. Bell DSH. Metformin-induced vitamin B12 deficiency can cause or worsen distal symmetrical, autonomic and cardiac neuropathy in the patient with diabetes. Diabetes, Obesity and Metabolism. 2022;24(8):1423-8.

13. Mumtaz H, Ghafoor B, Saghir H, Tariq M, Dahar K, Ali SH, et al. Association of Vitamin B12 deficiency with long-term PPIs use: A cohort study. Ann Med Surg (Lond). 2022;82:104762.

14. Gasmi A, Bjørklund G, Mujawdiya PK, Semenova Y, Peana M, Dosa A, et al. Micronutrients deficiences in patients after bariatric surgery. Eur J Nutr. 2022;61(1):55-67.

15. Bando T, Tokuda M, Katsuda I, Emi N, Tomita A. Involvement of folate and vitamin B12 deficiency in patients with normocytic anemia. Fujita Med J. 2023;9(2):134-41.