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Original Research Article

Association between Vitamin B12 Deficiency and Megaloblastic Anemia in Adults

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Abstract

Background: Vitamin B12 deficiency is a common reversible cause of megaloblastic anemia in adults, and this may be overlooked if neurological or non-specific symptoms predominate.

Methods: Cross sectional study of 150 adults with macrocytic anemia or suspected megaloblastic morphology. Serum levels of vitamin B12, folate, complete blood count, peripheral smear, lactate dehydrogenase and homocysteine were measured.

Results: Vitamin B12 deficiency (<200 pg/mL) was found in 96 patients (64.0%), folate deficiency in 18 (12.0%) and combined deficiency in 21 (14.0%). Morphological confirmation of megaloblastic anemia was obtained in 82/96 (85.4%) B12 deficient patients. Compared with non-B12-deficient patients, B12-deficient patients had lower hemoglobin (8.7 ± 1.5 g/dL vs. 10.1 ± 1.4 g/dL, p<0.001), higher MCV (112.6 ± 9.4 fL vs. 101.8 ± 6.7 fL, p<0.001), higher RDW (20.1 ± 3.2% vs. 16.8 ± 2.4%, p<0.001) and higher LDH (1280 ± 640 U/L vs. 520 ± 280 U/L, p<0.001). Serum B12 correlated inversely with MCV (r=-0.55, p<0.001) and homocysteine (r=-0.62, p<0.001).

Conclusion: Vitamin B12 deficiency was highly correlated with megaloblastic anemia and typical macrocytic, biochemical and clinical features in adults.

Keywords: Vitamin B12, Cobalamin, Megaloblastic Anemia, Macrocytosis, Homocysteine, Adult Anemia.

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Introduction

Megaloblastic anemia is macrocytic anemia due to failure of DNA synthesis in rapidly dividing hematopoietic precursors. The most common reversible causes are B12 and folate deficiencies; drugs, alcohol-related marrow toxicity, hypothyroidism, liver disease and myelodysplasia can mimic macrocytosis. Of these, vitamin B12 deficiency is clinically significant as neurological damage may be irreversible if treatment is delayed [1],[2]. Methionine synthase and methylmalonyl-CoA mutase reactions require vitamin B12. Deficiency results in ineffective erythropoiesis, intramedullary hemolysis, hypersegmented neutrophils, increased lactate dehydrogenase and increased homocysteine and increased methylmalonic acid. The clinical presentation may include fatigue, glossitis, paresthesia, gait disturbance, cognitive changes and mood symptoms [3],[4]. Vitamin B12 deficiency in adults can be caused by inadequate intake, vegetarian diet, pernicious anemia, gastric surgery, ileal disease, or exposure to medications, including proton-pump inhibitors and metformin, and pancreatic insufficiency. Subclinical and clinical B12 deficiency is common in India and other countries where the diet is mainly vegetarian [5],[6]. Often, diagnosis is not easy. In symptomatic patients, serum B12 might be at the low end of the normal range and macrocytosis may be absent if iron deficiency is present. So, clinicians frequently combine CBC indices, peripheral smear, biochemical markers and risk factors. This study examined the relationship between Vitamin B12 deficiency and megaloblastic anemia in adults and determined hematological and clinical markers of Vitamin B12 deficiency [7].

Materials and Methods

The study was cross sectional study carried out in the departments of Pathology and Medicine of a tertiary care teaching hospital. Macrocytic anemia (MCV >100 fL) and pancytopenia with macrocytosis were the criteria used to select adults aged 18 years or older with clinical suspicion of megaloblastic anemia. Patients who had received vitamin B12 or folate therapy in the last 3 months, those with known hematological malignancy, aplastic anemia, chronic liver failure, chronic kidney disease (on dialysis) and pregnancy were excluded. A total of 150 patients were included.

Demographic information, dietary pattern, gastrointestinal history, medication use, neurological symptoms and alcohol use were documented. Automated analyzer was used to perform CBC. Macro-ovalocytes, hypersegmented neutrophils, anisopoikilocytosis and cytopenias were evaluated in the peripheral smear. Serum vitamin B12, serum folate, lactate dehydrogenase and homocysteine were determined. Vitamin B12 deficiency was considered as serum B12 level <200 pg/mL with borderline levels being evaluated in the context of homocysteine and morphology.

The data were analyzed using SPSS version 26. Independent t-test or ANOVA were used to compare means. Chi-square test was used for analyzing categorical variables. Pearson correlation was used to examine the associations among serum B12 and hematological or biochemical variables. Predictors of B12 deficiency were identified using multivariable logistic regression. A p-value of < 0.05 was deemed to be significant.

Results

The mean age of the 150 participants was 44.2 ± 16.7 years, and 88 (58.7%) were male. Vitamin B12 deficiency was present in 96 patients (64.0%). Among B12-deficient patients, 82 (85.4%) had smear-confirmed megaloblastic anemia, 38 (39.6%) had pancytopenia and 31 (32.3%) had neurological symptoms.

Table 1: Clinical and laboratory comparison by vitamin B12 status.

Parameter	B12 deficient (n=96)	Not B12 deficient (n=54)	p-value
Age (years)	45.6 ± 16.4	41.7 ± 17.1	0.169
Hemoglobin (g/dL)	8.7 ± 1.5	10.1 ± 1.4	<0.001
MCV (fL)	112.6 ± 9.4	101.8 ± 6.7	<0.001
RDW (%)	20.1 ± 3.2	16.8 ± 2.4	<0.001
Platelets (×10^3/µL)	138 ± 82	198 ± 91	<0.001
LDH (U/L)	1280 ± 640	520 ± 280	<0.001
Homocysteine (µmol/L)	38.5 ± 16.4	17.2 ± 8.8	<0.001
Neurological symptoms, n (%)	31 (32.3)	6 (11.1)	0.004

Table 2: Peripheral smear findings among B12-deficient patients.

Smear finding	Number (n=96)	Percentage (%)
Macro-ovalocytes	84	87.5
Hypersegmented neutrophils	78	81.3
Anisopoikilocytosis	86	89.6
Pancytopenia	38	39.6
Leukopenia	44	45.8
Thrombocytopenia	51	53.1
Dimorphic picture	19	19.8

Table 3: Predictors of vitamin B12 deficiency in adults with macrocytic anemia.

Predictor	Adjusted odds ratio	95% CI	p-value
Vegetarian diet	2.86	1.45-5.64	0.002
Metformin use ≥1 year	2.41	1.08-5.38	0.032
PPI use ≥6 months	2.18	1.02-4.66	0.044
MCV ≥110 fL	3.92	1.96-7.85	<0.001
Hypersegmented neutrophils	4.15	2.03-8.49	<0.001
Neurological symptoms	2.74	1.16-6.47	0.021

Serum B12 level correlated inversely with MCV (r=-0.55, p<0.001), LDH (r=-0.49, p<0.001) and homocysteine (r=-0.62, p<0.001).

Hemoglobin improved by a mean of 1.8 ± 0.7 g/dL at four weeks in the subset of 64 B12-deficient patients with available follow-up. Neurological symptoms included paresthesia in 23 patients, gait imbalance in 7 and cognitive complaints in 5. Glossitis was documented in 29 patients (30.2%) with B12 deficiency.

Discussion

In this adult population, the most common cause of megaloblastic anemia was vitamin B12 deficiency. Lower hemoglobin, higher MCV, elevated RDW, elevated LDH and hyperhomocysteinemia were indicative of the association. These findings are in line with the mechanism of ineffective erythropoiesis and intramedullary hemolysis reported in clinical reviews [8,9].

Macro-ovalocytes and hypersegmented neutrophils were present in a large proportion of patients with B12 deficiency and were independent predictors after adjustment. The peripheral smear is thus of continuing clinical importance, especially in cases of a borderline B12 level, or when several deficiencies are present. Smear interpretation should be used in conjunction with biochemical tests as macrocytosis can be seen in liver disease, hypothyroidism, alcohol abuse and marrow disorders [10,11].

Patients with B12 deficiency had significantly more neurological symptoms. This is clinically significant because neurological involvement may occur in the absence of mild or severe anemia. Persistent neuropathy, gait impairment and neuropsychiatric complications can be prevented by early recognition and treatment [12].

Vegetarian diet was an independent predictor, due to the low natural cobalamin content of plant-based foods. Exposure to metformin and proton-pump inhibitors was also linked to deficiency, and periodic monitoring of these long-term medications is recommended, particularly when anemia, neuropathy or macrocytosis occurs [13,14].

The follow-up patients' response confirms the reversibility of hematological abnormalities following replacement. Oral high dose therapy may be effective in many patients but in severe neurological disease, pernicious anemia, malabsorption or very symptomatic presentations, intramuscular therapy is preferred [15].

The limitations are the lack of testing for methylmalonic acid and the lack of treatment follow-up for all participants. Not all laboratories offered tests for pernicious anemia antibody. However, the study shows a strong correlation between B12 deficiency and megaloblastic morphology in the routine clinical practice and simple predictors for early diagnosis [16,17]. It is important to note that there is dimorphic morphology in almost 20% of B12 deficient patients. Iron and B12 deficiency can occur together, which can decrease macrocytosis and cause an apparently normocytic anemia. In these patients, the use of RDW and smear review may be more helpful than just MCV. This is especially true in populations where nutritional deficiencies are overlapping. High LDH and homocysteine levels were biochemical markers of biochemical severity. Increased LDH is due to inadequate erythropoiesis and destruction of abnormal erythroid precursors in the marrow. Increased homocysteine levels is a marker of functional cobalamin deficiency, but not specific as increased homocysteine can also be caused by folate deficiency and renal dysfunction. Interpretation should therefore be kept in context.

History of medications was significant. Metformin could inhibit calcium-dependent ileal absorption of B12-intrinsic factor complex, and long-term acid suppression could decrease the release of food-bound B12-intrinsic factor complex. These mechanisms account for the fact that older people and diabetic patients on chronic treatment should be evaluated when macrocytosis, neuropathy or unexplained cytopenia is observed.

The level of clinical severity should be taken into account in making treatment decisions. If the anemia is severe, pancytopenia, or there are significant neurological symptoms or a possible malabsorption, replacement should be done as soon as possible and monitored closely. Reticulocytosis will occur in about 1 week and hemoglobin will improve within several weeks. Failure to respond should lead to re-evaluation of adherence, diagnosis, iron status, folate status and marrow disease.

The study also reminds the importance of preventative awareness. Patients often presented with vague symptoms for months prior to diagnosis. Patients often had vague symptoms for months before diagnosis. Avoidable morbidity can be prevented by simple dietary advice, screening of high-risk medication users and early testing in adults who are macrocytic or have neuropathy. Vitamin B12 deficiency is treatable, but if not recognized early, may result in permanent neurological damage [17].

The diagnostic difficulty is at the border of the normal range of vitamin B12. If this is the case, the finding of macro-ovalocytes, hypersegmented neutrophils, raised LDH, raised homocysteine and appropriate symptoms raises the likelihood of real deficiency. If available, repeat the assay or increase the concentration of methylmalonic acid can be helpful, but should not delay treatment in symptomatic patients with good clinical evidence.

In B12 deficiency, pancytopenia may mimic marrow failure or leukemia, especially if the patient is weak, has fever or bleeding. The diagnosis of megaloblastic morphology helps to avoid unnecessary anxiety and timely replacement. If cytopenias persist despite appropriate therapy, however, then a bone marrow evaluation is necessary to rule out myelodysplasia, aplastic anemia or hematological malignancy. Dietary history should be specific and not just vegetarian or non-vegetarian. Risk can be affected by frequency

of dairy consumption, egg consumption, affordability of animal-source foods, and chronic gastritis symptoms and history of gastrointestinal surgery. Such evaluation enables the clinician to customize the prevention and maintenance treatment following initial correction.

Diagnosis is as important as follow-up. Objective evidence of response is a rising reticulocyte count, improving platelet count and gradual reduction in MCV. Recovery from the neurological effects may be delayed and incomplete if symptoms were prolonged prior to treatment. Patient education should therefore focus on early reporting of numbness, imbalance and cognitive symptoms [17]. The high prevalence of thrombocytopenia and leukopenia in this group also highlights the need to consider B12 deficiency as a part of the differential diagnosis of unexplained cytopenias. When the clinical picture is compatible, a simple serum B12 assay and a review of the smear may obviate the need for an invasive investigation. Simultaneously, doctors should be careful when the cytopenia is severe, atypical cells are found or there is an incomplete response to treatment. A public health point of view, B12 deficiency is frequently underdiagnosed, as symptoms like fatigue, poor concentration and tingling are not specific. Early detection may be enhanced in patients with macrocytosis, chronic metformin use, chronic acid suppression, strict vegetarian diets or unexplained neuropathy, who are at risk for opportunistic screening. This is a cost-effective strategy to prevent disability that may occur after delayed treatment [17].

Conclusion: 64.0% of adults who were tested for macrocytic or megaloblastic anemia had B12 deficiency. Hemoglobin, MCV, elevated LDH, hyperhomocysteinemia, hypersegmented neutrophils and neurological symptoms were associated with B12 deficiency. Regular evaluation of dietary risk, medication exposure, CBC indices and peripheral smear may help to better facilitate timely diagnosis and treatment.

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