A Study of the Prevalence of Serum Vitamin B12 and Folic Acid Deficiency in Western Maharashtra

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ABSTRACT

Context: This study summarizes the prevalence of vitamin B12 and folic acid deficiency in the population coming to tertiary care center in Western Maharashtra along with the main presenting symptom routinely misinterpreted in daily practice. Aims and Objectives: 1. To study the prevalence of vitamin B12 and folic acid deficiency in the population of western Maharashtra. 2. To correlate the symptoms with serum vitamin B12 and folic acid levels. Materials and Methods: The present study is a cross-sectional observation study carried out on patients from western Maharashtra seeking medical attention on outpatient and inpatient basis in the medicine department of a teaching institute in Karad. One-hundred patients were selected on basis of below mentioned symptoms viz. tingling and numbness in extremities, dizziness, unsteady gait, early tiredness, forgetfulness, proximal weakness, distal weakness, chronic headache, less interest in work, chronic loose stools, strict vegetarians, alcoholics, intake of medications like anti-tubercular treatment, surgery involving terminal ileum. Serum vitamin B12 and folic acid levels of these patients were observed. Deficiency of vitamin B12 and folic acid was studied in 4 groups: (a) Absolute vitamin B12 deficiency; (b) Absolute folic acid deficiency; (c) Borderline vitamin B12 deficiency; (d) Combined vitamin B12 and folic acid deficiency. Results: Of the 100 cases, 33% patients were vegetarian. Folic acid deficiency formed the major chunk of deficiency group. Six percent patients had neuropsychiatric manifestations. Depressive illness in 1% patients, dementia in 0% patients, mania/hallucination in 0% patients each, and chronic headache in 1% patients. Neuropathy in form of loss of reflexes, decreased touch sensation was present in 9% patients. Posterior column involvement viz. Loss of joint position, vibration, positive Romberg’s sign were present in 34% patients of vitamin B12 and folic acid deficiency. Conclusion: In a small study, it was found that megaloblastic anemia may have symptoms and signs referable to several systems including hematology, dermatology, gastrointestinal, neurology, and neuropsychiatry.

Keywords: Folic acid, hematological manifestations of folic acid deficiency, hematological manifestations of vitamin B12 deficiency, vitamin B12

Introduction

Megaloblastic anemias are a heterogenous group of disorders that share common morphologic characteristics. Erythrocytes are larger and have heavier nuclear-to-cytoplasmic ratios compared to normoblastic cells. Neutrophils can be hypersegmented, and megakaryocytes are abnormal. On the molecular level in the megaloblastic cells, the maturation of nuclei is delayed while cytoplasmic development is normal. Megaloblastosis is a generalized disorder because non-hematopoietic cells such as GI and uterine cervical mucosal cells can also have megaloblastic features. The etiology of megaloblastic anemias is diverse, but a common basis is impaired DNA synthesis. The most common causes of megaloblastosis are cobalamin (vitamin B12) and folate deficiencies. The most well-known causes of cobalamin deficiency are pernicious anemia, failure of absorption of cobalamin in the terminal ileum, effects of medications e.g., Metformin interferes with vitamin B12 absorption. The development of megaloblastic anemia is usually insidious; therefore, patients are often relatively asymptomatic because they have had time to adjust to the marked fall in hemoglobin levels. Shorvon et al. stated that there is no relation between hematological and neurological abnormalities. There are a number of sporadic reports in the literature of compromised cellular immune status in patients with cobalamin and folate deficiencies affecting neutrophil functions. Despite being studied so much, these deficiencies are routinely missed in clinical practice leading to misdiagnosis and mismanagement. We hereby aim to find out the prevalence of these deficiencies in general population and correlate the findings with the symptomatology.

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Materials and Methods

The present study is a cross-sectional observation study carried out on patients seeking medical attention on outpatient and inpatient basis in the medicine department of a teaching institute of Krishna Institute of Medical Sciences University, Karad. Large number of patients were studied, and out of them, 100 patients were selected on basis of below mentioned symptoms viz. history of tingling and numbness in extremities, dizziness, unsteady gait, early tiredness, forgetfulness, proximal weakness, distal weakness, chronic headache, less interest in work, chronic loose stools, strict vegetarians, alcoholics, intake of medications like anti-tuberculor treatment, surgery involving terminal ileum were included in this study.

Exclusion criteria
- Normocytic normochromic peripheral blood smear
- Only microcytic peripheral blood smear
- History of bleeding from any site
- Other obvious structural and metabolic diseases.

Serum/heparinized plasma from patients were collected, and vitamin B12 and folic acid levels were determined using automated chemiluminescence system where the vitamin B12 from patient’s serum competes with vitamin B12 labeled with acridinium ester in the Lite Reagent, for a limited amount of purified intrinsic factor, which is covalently coupled to paramagnetic particles in solid phase. The assay uses releasing agent (Sodium Hydroxide) to release vitamin B22 from the endogenous binding proteins in the samples and prevents rebinding after the solid phase is added to the sample. The folic acid levels were determined using ACS: 180 folate assay using direct chemiluminescent technology. Folate in the patient’s sample competes with acridinium ester-labeled folate in the Lite Reagent for a limited amount of biotin-labeled folate binding protein. Biotin-labeled folate binding protein binds to avidin that is covalently coupled to paramagnetic particles in the solid phase. In the ACS: 180 folate assay, the sample is pre-treated to release folate from endogenous binding proteins in the sample.

Results

One-hundred cases of suspected vitamin B12 and folic acid deficiency were studied:
- There were 33% patients who were vegetarian [Table 1]
- Major cause of vitamin B12 deficiency was found to be folic acid deficiency
- Deficiency of vitamin B12 and folic acid was studied in 4 groups
  - Absolute vitamin B12 deficiency (S. Vitamin B12 <200 pg/ml)
  - Absolute folic acid deficiency (S. Folic acid <5 ng/ml)
  - Borderline vitamin B12 deficiency (S. Vitamin B12-200-250 pg/ml)
  - Combined vitamin B12 and folic acid deficiency.

Of the tested 100 patients [Figure 1]
- Absolute vitamin B12 deficiency was found in 6% patients
- Borderline vitamin B12 deficiency was found in 9% patients
- Absolute folic acid deficiency was found in 26% patients
- Combined vitamin B12 and folic acid deficiency was found in 10% patients.

Thus, folic acid deficiency formed the major chunk of deficiency group.

The clinical features in megaloblastic anemia were found to be tingling numbness in 11% patients, unsteady gait in 3% patients, giddiness in 41% patients [Figure 2], pallor in 85% patients, diffuse hyperpigmentation on skin in 52% patients, glossitis in 40% patients, and stomatitis in 57% patients [Figure 3].

MCV was raised in 23% patients, 37% patients had macrocytosis, and 6% patients had hypersegmented neutrophils on peripheral blood smear [Figure 4] and 8% patients had thrombocytopenia.

Twenty-eight percent patients had low RBC count i.e., <3 × 10⁶ eu/mm

Six percent patients had neuropsychiatric manifestations. Depressive illness in 1% patients, dementia in 0% patients, forgetfulness in 1% patients, mania/hallucination in 0% patients each, and chronic headache in 1% patients.

Neuropathy [Tables 2 and 3] in form of loss of reflexes, decreased touch sensation was present in 9% patients. Posterior
column involvement viz. Loss of joint position, vibration, positive Romberg’s sign were present in 34% patients of vitamin B12 and folic acid deficiency.

**Discussion**

Megaloblastic anemias are disorders caused by impaired DNA synthesis and characterized by presence of megaloblastic cells, the morphologic hallmark of this group of anemia. The unclear basis for megaloblastosis is a failure in the synthesis and assembly of DNA. The most common causes of megaloblastosis are cobalamin and folate deficiencies. A hallmark of megaloblastic anemia is ineffective erythropoiesis, as evidenced by erythroid hyperplasia in the bone marrow, a decreased peripheral reticulocyte count, and an elevation in lactate dehydrogenase (LDH) and indirect bilirubin levels.

The pathogenesis of these findings is the intramedullary destruction of fragile and abnormal megaloblastic erythroid precursors. Causes of vitamin B12 deficiency include nutritional deficiency, food-cobalamin malabsorption, pernicious anemia, gastrectomy, Zollinger- Ellison Syndrome, Blind Loop Syndrome, fish tapeworm, and Nitrous Oxide. Cause of folate deficiency includes dietary folate depletion, impaired absorption viz. metabolic bone disease or bleeding due to deficiencies in vitamin K-dependent factors; tropical sprue, regional enteritis, intestinal lymphoma, surgical intestinal resection, amyloidosis, Whipple’s disease, scleroderma, phenoxytoin therapy, psoriasis and exfoliative dermatitis, hyperalimentation, hemodialysis, drugs (methotrexate,
acetylcholine, 5FU, hydroxyurea, Phenobarbital, oral contraceptives, metformin, omeprazole, etc.)

These deficiencies are readily diagnosed on peripheral blood smear, which shows oval macrocytes, hypersegmented granulocytes, and anisopoikilocytosis. In severe anemia, red blood cell inclusions may include Howell-Jolly bodies, Cabot rings, and punctate basophilia. The macrocytosis can be obscured by the co-existence of iron deficiency, thalassemia minor or inflammatory disease.

This study has evaluated the prevalence of serum vitamin B12 and folic acid deficiencies by the means of clinical examination supported by the laboratory data. B12 and folic acid deficiency are the important health problems in many countries worldwide although the prevalence of deficiency shows a high variability. Even though the human body has enough vitamin B12 stores to last for up to five years, its deficiency is not uncommon. The main systems affected due to vitamin B12 deficiency are the hematological, skin and mucous membranes, and the nervous system. Neurological features are attributable to pathology in the peripheral nerves, optic nerves, posterior and lateral columns of the spinal cord and brain. Vitamin B12 deficiency is a classic neurological ‘system-specific degeneration,’ in which particular sets of neurons are affected because of their selective vulnerability. An increased prevalence of vitamin B12 deficiency has been reported in patients infected with the human immunodeficiency virus (HIV). However, none of our patients had HIV infection. Vitamin B12 deficiency causes a wide spectrum of neurological manifestations ranging from neural tube defects to changes in cognition and behavior. Many unusual manifestations have been reported in infants and adults including movement disorders and seizures.

Vegetarians constituted 33% of the study population, which is contrary to the earlier Indian studies, which have shown that the vegetarians are at a higher risk of B12 and folic acid deficiencies. Vegetarians are at a higher risk of developing vitamin B12 deficiency, which is probably corroborated by the finding that vegetarians had a statistically more severe form of megaloblastic changes in the bone-marrow as compared to the non-vegetarians. The UK Vegan Society, the Vegetarian Resource Group, and the Physicians Committee for Responsible Medicine, among others, deny that non-animal food sources of vitamin B12 are reliable and recommend that every vegan who is not supplementing consume B12-fortified foods. Myeloneuropathy was the commonest neurological manifestation in this study. In another similar study from the West, diminished vibratory sensation and proprioception in the lower extremities were the most common objective neurological findings. This difference may be due to the delayed presentation of patients to our center in this study.

Tingling numbness and fatigue were the commonest symptom in this series. Vitamin B12 deficiency has been implicated in conditions where fatigue is a prominent symptom such as chronic fatigue syndrome. In this study, fatigue was associated with a low Hb level.

Hyperpigmentation over the extremities, especially over the dorsum of the hands with accentuation over the terminal phalanges and inter-phalangeal joints, associated with pigmentation of the oral mucosa, is characteristic of vitamin B12 deficiency. These findings resolve with the supplemental therapy. The association between neuropsychiatric disorders and vitamin B12 deficiency is well-known. Dementia in our study did not correlate with either the duration of symptoms or the severity of the megaloblastic changes in the peripheral smear. These results are similar to the findings reported by Lindenbaum et al. where it was shown that the neuropsychiatric disorders due to cobalamin deficiency occurred commonly in the absence of anemia or macrocytosis.

A high prevalence of low serum vitamin B12 levels and other indicators of vitamin B12 deficiency have been reported among people with Alzheimer’s disease and older people. However, evidence of any efficacy of vitamin B12 therapy in improving the cognitive function of people with dementia and low serum B12 levels is insufficient.

Many different studies have tried to describe a possible consequence of the combined defect of vitamin B12 and folate. Riggs et al. investigated the relations between plasma concentrations of folate, vitamin B12, vitamin B6, and homocysteine and scores on a battery of cognitive tests in 70 men, aged 54-81, participating in the Normative Aging Study. Lower folate and vitamin B12 concentrations were associated with poorer spatial copying skills. In addition, plasma homocysteine concentration, which is inversely correlated with plasma folate and vitamin B12 concentrations, was a stronger positive predictor of spatial copying performance than either folate or vitamin B12 concentrations. Among markers of cobalamin/folate status, plasma homocysteine shows the best association with neuropsychiatric dysfunction. Unfortunately, we were not able to investigate our group of patients for homocysteine levels due to financial status of the patient as most of our study-group patients were from very low socio-economic status.

Stomatitis was found in 57% patients in our study, which was persistent with the findings of the study by Miller et al. and Koybasi et al., and this was found to be due to higher incidence of recurrent aphthous ulcers in cases that have vitamin B12 deficiency suggest a direct role of this vitamin on the pathogenesis of aphthous ulcers. Research has established the effectiveness of different routes of B12 administration, primarily intranasal and sublingual dosing, but neither has been proven to be superior to oral dosing; recommendations are based on a consumer’s individual circumstances.

A recent work examined the relationship between low levels of serum vitamin B12 and folic acid and cognitive functioning in very old age. In general, the effects of folic acid exceeded those of B12, and these findings are persistent with our study group patients. Quite different results emerged from Jadhav et al. They
were no clear signs of slowing the progression of dementia, when treating patients with subnormal serum levels, with supplements of vitamin B12. Neither vitamin B12 nor folic acid supplementation\(^{25}\) affected recognition or primary memory in very old age, even though the subjects with low folic acid levels showed impairment in both word recall and object recall.

On a close scrutiny, the results of our present study are similar to and not grossly different from the previous studies, thus proving that virtually any system can be affected by such deficiencies and a strong suspicion can prevent many wrong diagnoses leading to avoidance of unnecessary investigations to diagnose and treat a patient with the above mentioned symptoms. For the primary health care physicians, these deficiencies being the commonest causes of general symptomatology of patients, suspecting and treating the same can avoid many late-consequences at the primary level itself and leads to avoidance of many unnecessary investigations, thus proving a cost-effective management, especially in our country.

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