Clinical Interpretations of the Measurement of Folic Acid and Vitamin B₁₂ in Neuromuscular Disease

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ABSTRACT

Fifty-six patients were studied in an attempt to define neurologic complications associated with folic acid and vitamin B₁₂ deficiency. Two patients had abnormal levels of vitamin B₁₂. One of these showed decreased vibratory sense in the legs. Ten of 15 patients with serum folate less than 4.0 ng per ml and 16 of 23 patients with serum folate less than 6.0 ng per ml by radioassay had neurologic abnormalities. The control group demonstrated 17/31 with neurologic abnormalities, a figure not significantly different from the deficient group. It is suggested that more patients with "pure folate deficiency" must be analyzed to eliminate the possible role of toxic effects of alcohol and other diseases of the nervous system in patients with low serum folate and neurologic problems.

Introduction

It is well established that vitamin B₁₂ deficiency is associated with various neurological complications including peripheral neuropathy, subacute combined systems disease and, less commonly, neuropsychiatric manifestations. Folic acid deficiency was not recognized as being associated with any neurological disease until recent years. In fact, folic acid was thought to reverse the megaloblastic bone marrow changes of B₁₂ deficiency, while allowing the neurologic complication of B₁₂ deficiency. Folic acid deficiency is commonly associated with a poor diet and chronic alcoholism, neurologic abnormalities in these circumstances are usually thought to be related to the more direct effects of alcohol on the nervous system.

In 1965 Grant et al. pointed to folic acid deficiency as the potential reason for neurologic problems in a group of nutritionally deprived individuals. Additionally, a number of patients being treated with diphenylhydantoin for epilepsy were observed to develop megaloblastic anemia and neuropathy unresponsive to vitamin B₁₂ but responsive to folic acid. Many of these patients were discovered to have low levels of both vitamin B₁₂ and folate in the serum. Proof that isolated deficiency of folic acid can produce neurologic damage is lacking.

Strachan and Henderson in 1967 described two patients with advanced dementia and megaloblastic anemia due to folate deficiency. Folate therapy pro-
duced a marked improvement in the mental state. Recently, Reynolds and coworkers\(^9\) reopened the question of neurologic disease associated with folate deficiency. Twenty-four patients with folate levels less than 3 ng per ml were studied. For controls, patients chosen were matched for age and sex with folic acid levels over 6.9 ng per ml. A statistically significant increase in organic brain syndrome was discovered as was positive Babinski response in the folate deficient patients. In their series, 17/24 patients with low folate levels had evidence of organic brain syndrome. Also 5/24 folate deficient patients had a Babinski reflex while none of the patients with normal folate had a positive Babinski response. These investigators showed that peripheral neuropathy was more common in the alcoholic patients in both folate deficient and non-deficient patients, but that evidence of organic brain syndrome was more frequently seen in patients with low folate and did not correlate with a history of excessive intake of alcohol. It was concluded that consideration must be given to the possibility that nutritional deficiency of folate may result in neurological complications.

To review the possible role of folate and vitamin B\(_{12}\) in neuromuscular disease, the present authors have studied a series of anemic patients in whom vitamin B\(_{12}\) and folate in the serum was measured.

**Methods**

Fifty-six anemic patients in whom vitamin B\(_{12}\) and folate levels were measured upon admission into the hospital were studied to determine the presence or absence of neurologic findings. Anemia was defined as hct < 35 percent or hb < 12 g per dl. Review of the medical history and physical findings was carried out by one investigator, (B.M.) without knowledge of the serum levels of vitamin B\(_{12}\) and folic acid.

Vitamin B\(_{12}\) and folic acid were measured by radioassay.\(^7,8,11,13\) Serum was frozen immediately after collection. Normal controls were sera from Red Cross blood donors. A pool of 20 sera was used for daily quality control and to determine replication of the assays.

After patient data had been collected and the assays had been completed, an attempt to find correlations between neurologic abnormalities and vitamin B\(_{12}\) and folic acid levels was made by the other investigator. (E.M.)

**Results**

Fifty normal sera were examined in duplicate and produced a mean normal value for folic acid of 12 ± 4 ng per ml and for vitamin B\(_{12}\) of 650 ± 175 pg per ml. Thirty replicate determinations on the same pool of normal sera produced a coefficient of variation of 13 percent for vitamin B\(_{12}\) and 17 percent for folic acid. The lower limit of normal for vitamin B\(_{12}\) was taken as 300 pg per ml with a normal range to 1000 pg per ml. For folic acid, the lower limit was 4.0 ng per ml with a normal range to 20 ng per ml. The patients ranged in age from 7 to 91 with a mean of 47 years. the male:female ratio was 38/18. Of the 56 anemic patients reviewed, 15 had folic acid levels less than 4.0 ng per ml and two had vitamin B\(_{12}\) levels less than 300 pg per ml. There were eight patients with folic acid levels between 4.0 and 6.0 ng per ml which were analyzed separately.

There were approximately twice as many men as women in each of the three groups determined by their folic acid levels. Both male and female groups were found to be equally distributed between those with neurological findings and those without. No statistically significant difference of age or sex distribution occurred in any of the groups. Of the two patients with low vitamin B\(_{12}\) levels,
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neither had peripheral blood macrocytosis and bone marrow examinations were not performed. One had a normal neurologic examination. The other had loss of vibratory sense in the lower leg. These patients are not considered further.

Of 15 patients with low folic acid levels, 11 had peripheral blood macrocytosis (seven with mean corpuscular volume (MCV) greater than 100, four with macrocytes described on smear). Two had bone marrow examinations, both of which showed moderate to marked megaloblastic changes. Of eight patients with intermediate values of folic acid, six had peripheral blood macrocytosis (four with MCV greater than 100, two with macrocytes described on smear). In the group of patients with normal levels of vitamin B₁₂ and folic acid, 14 showed peripheral blood macrocytosis (nine with MCV greater than 100, five with macrocytes described on smear). Seven bone marrow examinations were done and three showed slight to moderate megaloblastic changes. Four of the five patients with megaloblastic changes in the bone marrow had peripheral blood macrocytosis, but three of the four patients who had no evidence of megaloblastic changes in the marrow also had peripheral blood macrocytosis. Only seven of 29 patients who had peripheral blood macrocytosis had bone marrow examinations performed. These results were statistically insignificant.

Examination of the relationship between folic acid levels and neurologic disease revealed neurologic abnormalities in 10 out of 15 patients (67 percent) with low folic acid. However, neurologic abnormalities were also found in 23 of 39 patients who had folic acid levels greater than 4.0 ng per ml (59 percent) as shown in table I. There were eight patients whose folic acid levels were between 4.0 and 6.0 ng per ml, a level which was considered by us suspicious for folic acid deficiency. Six of the eight had neurologic abnormalities. None of these interrelationships proved to be statistically significant using X² method.

Table I

<table>
<thead>
<tr>
<th>Folic Acid ng per ml</th>
<th>Neurologic Disease</th>
<th>No Evidence of Neurologic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;6.0</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>4.0 - 6.0</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>&lt;4.0</td>
<td>10</td>
<td>5</td>
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Examination of the relationship between neurologic abnormalities found and the folic acid level revealed more instances in each category in the patients with normal levels of folic acid than in those with low folic acid (table II). Neurologic abnormalities were significantly correlated with macrocytosis p < 0.02 (table III).

Discussion

In this series of patients, correlation between serum levels of folic acid and vitamin B₁₂ and neurologic findings were difficult to demonstrate. Of our series of anemic patients, only two (less than four percent) demonstrated low vitamin B₁₂ while 15 (27 percent) demonstrated low folic acid. The number of folate deficient patients increases to 23 (41 percent) if the borderline group with folic acid measurements between 4.0 and 6.0 ng per ml is included with the abnormals.

These findings strongly suggest that while nutritional deficiency in this population of patients may account for a substantial part of the blood and neurologic abnormalities seen, deficiency of vitamin B₁₂ or folic acid as determined by serum level does not provide the entire explanation. Although Reynolds et al⁹ showed a higher incidence of chronic brain syndrome and extrapyramidal signs in their
folic acid deficient group, that finding was unable to be confirmed by us even when including patients with folate levels between 4.0 and 6.0 ng per ml. It would be enlightening to know what the neurologic status was in Reynolds' patients with folate levels between 3 ng per ml and 6.9 ng per ml.

On the other hand, the choice of controls matched only by age and sex in the Yale study may have introduced a bias by selecting hematologically normal individuals who would have much less chance of having neurological disease from other nutritional deficiencies. While the patients were analyzed for a history of alcoholism, manifestations of anemia or other nutritional abnormalities to match the "folate deficient" patients with the controls were not included.

Our study suggests a strong relationship between macrocytosis in the peripheral blood and neurological disease. Whether the macrocytosis should be interpreted as a manifestation of folate deficiency, which could not be measured by serum levels, or a manifestation of other nutritional deficiency, also associated with neurologic abnormalities, is a moot point. A study of tissue (red blood cell) folate would be of interest and will be undertaken, since serum folate levels fluctuate more readily than tissue folate levels do.5

In a population where alcoholism and other neurologic problems may be superimposed on nutritional deficiency, it is difficult to differentiate the effects owing to folate deficiency. Until larger numbers of patients have been studied without these interfering factors, the association of neurologic disease with pure folic acid deficiency remains but an intriguing speculation.

References


