Methylmalonic Acid Concentration in Serum Not Affected in Hepatic Disease

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Accumulation of methylmalonic acid may provide an early clue to deficiency of cobalamin (vitamin B12) in tissue. Metabolic abnormalities involving precursors of methylmalonic acid are frequently observed in patients with hepatic diseases. To establish whether methylmalonic acid accumulates and thereby gives false-positive test results for cobalamin deficiency, we measured the concentration of methylmalonic acid in serum of patients with various hepatic diseases. Many of the patients had increased concentrations of cobalamin in serum. In serum from 70 patients, the mean concentration of methylmalonic acid (252, SE 25 nmol/L) did not differ significantly from that found in healthy subjects (211, SE 12 nmol/L). We conclude that the assay of methylmalonic acid in serum may be useful for evaluating cobalamin status in hepatic disease with functional cobalamin deficiency despite an artificially increased normal or high concentration of cobalamin in serum.

Additional Keyphrases: cobalamin • vitamin B12

Clinical cobalamin deficiency is much more diverse than previously believed (1, 2). Subtle cobalamin deficiency is clinically significant, and the limitations of using only the serum cobalamin concentration to detect cobalamin deficiency are widely recognized (3–10). Moreover, the concentration of cobalamin in serum does not necessarily reflect the cobalamin status of the whole body.

Patients with cobalamin deficiency have increased concentrations of methylmalonic acid (MMA) in serum because 5′-deoxynucleotidecobalamin is required for the enzymatic conversion of L-methylmalonyl-CoA to succinyl-CoA by methylmalonyl-CoA mutase (EC 5.4.99.2). The clinical importance of determining the concentration of MMA in serum has been a subject of increasing interest during the past few years (11, 12). It is important to know if test results for individuals with various diseases differ from those for healthy subjects (Phase II trials).

Important precursors of L-methylmalonyl-CoA include the amino acids isoleucine, valine, threonine, and methionine. Because the liver plays a central role in amino acid metabolism, we undertook this study to establish whether hepatic disease complicates the measurement of MMA in serum.

Materials and Methods

In a previous study (10), we established a central 95th percentile reference interval for MMA in serum from 58 healthy subjects [age range: 40–68 years (median 53); 37 women, ages 40–64 years (median 48), and 21 men, ages 43–68 years (median 53). All subjects had normal concentrations of cobalamin and creatinine in serum. Erythrocyte counts and mean erythrocyte cell volume were within normal limits, as were results of liver-function tests. No subject took drugs known to interfere with cobalamin metabolism.

The study group comprised 70 patients with several hepatic diseases (19 with various hepatic disorders such as tumors, cholestasis, or different infections with septicemia; 19 with acute hepatitis; 17 with active chronic hepatitis; and 15 with liver cirrhosis). Exclusion criteria were subnormal concentrations of cobalamin or increased concentrations of creatinine in serum. All subjects were patients from the University Department of Infectious Diseases at Marselisborg Hospital. Diagnoses of hepatic diseases were based on clinical and laboratory data and pathological findings of liver biopsies. Table 1 lists some characteristics of the patients studied.

Blood samples for assessing MMA were taken from an antecubital vein. After the blood coagulated at room temperature for 1 h, we separated the serum by centrifugation and stored it at −20 °C.

MMA in serum was measured by stable-isotope dilution with solid-phase extraction of the sample (13). The total analytical imprecision of our method (SD) is 32 nmol/L at a concentration in serum of 350 nmol/L (10).

Comparisons between patients and control subjects were made with Mann–Whitney's two-tailed U-test. Significance was at P <0.05.

Results

The mean concentration of MMA in serum from the 70 patients studied (252, SE 25 nmol/L) did not differ significantly from that found for 58 healthy subjects (211, SE 12 nmol/L).

The ranges of concentrations of MMA in the four diagnostic groups (various hepatic disorders, acute hepatitis, active chronic hepatitis, and liver cirrhosis) are shown in Figure 1. The median concentrations of MMA for these groups were 265, 167, 267, and 285 nmol/L, respectively. In comparison, in our normal control subjects the median concentration was 198 nmol/L. Concentrations of MMA in serum were significantly lower in patients with acute hepatitis than in the other three groups.

We correlated MMA concentration to the hematological data and liver-function tests listed in Table 1. The only significant correlation was obtained between the

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Received October 30, 1991; accepted January 31, 1992.
Table 1. Comparison of Healthy Control Subjects and Subgroups of Patients with Hepatic Diseases

<table>
<thead>
<tr>
<th>Patients with hepatic diseases</th>
<th>Healthy control subjects (n = 50)</th>
<th>Various hepatic disorders (n = 19)</th>
<th>Acute hepatitis (n = 19)</th>
<th>Chronic hepatitis (active) (n = 17)</th>
<th>Liver cirrhosis (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, ♂/♀</td>
<td>37/21</td>
<td>9/10</td>
<td>8/11</td>
<td>9/8</td>
<td>7/8</td>
</tr>
<tr>
<td>Age, years</td>
<td>40–68*</td>
<td>18–85</td>
<td>18–62</td>
<td>9–75</td>
<td>26–86</td>
</tr>
<tr>
<td>Blood hemoglobin, mmol/L</td>
<td>6.2–10.5</td>
<td>6.3–10.1</td>
<td>5.0–10.9</td>
<td>7.3–10.2</td>
<td>5.1–9.5</td>
</tr>
<tr>
<td>Erythrocyte mean cell volume, fl</td>
<td>74–100</td>
<td>86–110</td>
<td>83–103</td>
<td>87–110</td>
<td>84–113</td>
</tr>
<tr>
<td>Serum cobalamin, pmol/L</td>
<td>159–589</td>
<td>205–3520</td>
<td>222–6470</td>
<td>218–933</td>
<td>305–890</td>
</tr>
<tr>
<td>Serum folate, mmol/L</td>
<td>1.9–11.8</td>
<td>1.4–21.2</td>
<td>1.9–27.7</td>
<td>2.6–10.9</td>
<td>0.9–8.3</td>
</tr>
<tr>
<td>Serum total bilirubin, μmol/L</td>
<td>4–15</td>
<td>2–228</td>
<td>3–246</td>
<td>3–103</td>
<td>11–190</td>
</tr>
<tr>
<td>Serum alkaline phosphatase, U/L</td>
<td>77–275</td>
<td>120–2990</td>
<td>180–3410</td>
<td>142–928</td>
<td>142–2045</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>[36–50]*</td>
<td>21–42</td>
<td>24–43</td>
<td>32–41</td>
<td>15–42</td>
</tr>
<tr>
<td>Prothrombin time index</td>
<td>[0.80–1.20]*</td>
<td>0.15–1.17</td>
<td>0.41–1.30</td>
<td>0.57–1.30</td>
<td>0.17–1.03</td>
</tr>
</tbody>
</table>

* Range (and median).

- Not measured in healthy central subjects. Ranges indicate normal central 95 percentiles reference intervals for our laboratory.

![Fig. 1. Concentrations of methylmalonic acid in the different groups of patients.](image)

The shaded area indicates reference interval (based on results for 58 healthy subjects); each point is the mean of duplicate determinations.

cobalamin assay and determination of MMA in serum (r = -0.21). No age- or sex-related differences were apparent.

Discussion

The importance of measuring MMA in serum as an aid to diagnosing early cobalamin deficiency is well established. A prerequisite for interpreting MMA measurements is knowledge of the sources of variation and the diagnostic specificity of the MMA assay. Our laboratory has focused on the factors that may affect concentration of MMA. The analytical performance of the MMA assay (13), the biological variability (10), the lack of dietary influences (14), and the reference interval for serum MMA in healthy individuals are now established (10). In a previous study we reported increased concentrations of MMA during renal insufficiency (15).

Our goal here was to evaluate the possible influence of hepatic disease on MMA in serum. Metabolic abnormalities involving free amino acids, particularly branched-chain amino acids (valine, isoleucine, and leucine), are frequently observed in patients with hepatic disorders. Recently, Azuma et al. (16) reported that a decrease in branched-chain amino acids in serum paralleled the severity of hepatic parenchymal damage. Valine and isoleucine, the two most important precursors of L-methylmalonyl-CoA, are mainly metabolized in skeletal muscle. However, their concentrations in serum are considerably affected by the serum insulin concentration, which is related to liver functions (17).

To our knowledge, no investigation of the effect of hepatic disorder on concentrations of MMA in blood has been reported, but earlier investigators, applying various colorimetric and chromatographic methods of analysis, noted that urinary excretion of MMA was within normal limits in patients with hepatic cirrhosis (18–20). In accord with this, we observed normal results in all but 3 of the 15 patients with hepatic cirrhosis. We have no straightforward explanation for the increased concentration in these three patients. We could hypothesize that these patients might have been deficient in cobalamin; if so, the concentration of MMA in serum should decrease substantially after vitamin B12 therapy. However, two of these patients died before follow-up appointments, and, for reasons unknown, the third presented with a normal MMA concentration in serum after three months (and on several later occasions), although no vitamin B12 was administered.

Table 1 shows that many of the patients had increased concentrations of cobalamin in serum. In liver disease, increases in the plasma transcobalamin concentrations, particularly transcobalamin I, lead to concomitant in-
creases of the plasma cobalamins (21). An additional factor in some liver diseases, especially in acute hepatitis, may be the release of cobalamin from damaged hepatic cells into the blood. Thus the decreased MMA seen in serum of patients with acute hepatitis could occur because of a surplus of 5'-deoxyadenosylcobalamin at the site of biochemical utilization (e.g., the mitochondria in muscle cells). However, in acute hepatitis only a weak correlation was obtained between the cobalamin assay and determination of MMA in serum ($r = -0.12$); furthermore, the MMA concentrations in the eight patients with increased cobalamin concentrations in serum and in the 11 patients with normal cobalamin concentrations did not differ significantly. We speculate that the decreased serum concentrations of MMA in acute hepatitis may have been caused by reduced protein intake.

In conclusion, our main finding is that hepatic disease per se is not associated with an increased concentration of MMA in serum. This finding indicates that the assay of serum MMA may be useful for evaluating cobalamin status in patients with functional cobalamin deficiency despite an artificially increased normal or high cobalamin concentration in serum caused by abnormalities in cobalamin-binding proteins.

References