Cobalamin (Vitamin B₁₂) Deficiency Detection by Urinary Methylmalonic Acid Quantitation

By Eric J. Norman, O. J. Martelo, and M. Drue Denton

A study was made to assess the value of cobalamin deficiency detection through quantitation of urinary methylmalonic acid (MMA). Urinary MMA was measured in 1118 patients suffering from megaloblastic anemia, other anemias, elevated red cell mean corpuscular volume, or unexplained neurologic disorders. Patients without proven cobalamin deficiency had urinary MMA levels <20 µg/ml. All patients (n = 27) confirmed to have cobalamin deficiency showed MMA levels >20 µg/ml. Data are presented showing the Schilling test results, the comparison of serum cobalamin to urinary MMA levels, and other basic hematologic data. MMA levels are a good indication of cobalamin distribution and function since they are directly related to a cobalamin-dependent metabolic pathway. With rapid, reliable quantitation by mass spectrometry, urinary MMA can now be a useful clinical test.

Patients with cobalamin deficiency have elevated levels of urinary methylmalonic acid (MMA) since cobalamin is required for conversion of MMA to succinic acid. Although it has been known since 1967 that MMA levels usually correlate with reduced serum cobalamin levels, the quantitation of MMA has not been done routinely. This is primarily because no method that was both sensitive and rapid has existed. When normal levels of MMA were quantitated, the method was laborious and time consuming and has not been adopted as a clinical procedure. Therefore, recent studies on urinary MMA have used methods that were unable to detect normal levels of MMA.

Our laboratory has recently developed a mass spectrometric assay for urinary MMA that is rapid, sensitive, and reproducible. The method is noninvasive and requires only a random urine specimen. Although the mass spectrometer is not now widely available in most hematology laboratories, most medical center hospitals do have one that is usually located in a toxicology, pharmacology, or pediatrics laboratory.

Determinations of urinary MMA have become increasingly important because recent reports have indicated that the serum cobalamin radioassay can give false high values. This may be due to an artifact caused by using an R binder protein rather than the more specific intrinsic factor. False high values can also result from patients taking chloral hydrate as a sedative.

Furthermore, serum cobalamin levels may be normal or high despite a functional cobalamin deficiency. Serum cobalamin and cobalamin-binding proteins have been shown to be abnormally high in some hematologic malignancies such as acute promyelocytic leukemia and chronic myeloid leukemia. In addition, a study of nonhematologic malignancy showed approximately 50% of these patients had abnormalities of cobalamin and its binding protein. In situations where the serum cobalamin level is artificially elevated due to abnormalities in cobalamin-binding protein, measuring cobalamin status requires either a tissue biopsy for cobalamin content or a laboratory test for normality of function of a cobalamin-dependent pathway, such as measurement of MMA, or the deoxyuridine (du) suppression test. Excretion of elevated MMA has been reported to be the first indication of cobalamin deficiency since it is a reflection of the tissue availability of the vitamin.

Low serum cobalamin levels in the absence of tissue deficiency of the vitamin have been reported in a variety of conditions, sometimes as an artifact of the assay procedure, sometimes because of abnormality of a cobalamin-binding protein, and sometimes without clear explanation.

To help avoid problems in the diagnosis of cobalamin deficiency, quantitation of urinary MMA may be very helpful. We report data on 1118 patients who were assayed for urinary MMA.

Materials and Methods

Specimen Storage

Urine specimens were stored without preservative in plastic tubes at −20°C. Although urines were frozen upon receipt, no special handling was required. No significant changes in MMA levels were noted in several urines stored at room temperatures for 6 mo containing both high and normal MMA levels.

MMA Measurements

The method of assaying for urinary MMA has been previously described in detail. Urine (50µl) was derivatized to form the dicyclohexyl ester of MMA and quantitated using a Finnigan 9500...
Table 1. Range of Concentrations of Urinary MMA in Patients

<table>
<thead>
<tr>
<th>MMA (μg/mL Urine)</th>
<th>Number of Patients (n = 1118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1-1.0</td>
<td>281</td>
</tr>
<tr>
<td>1-2.0</td>
<td>335</td>
</tr>
<tr>
<td>2-3.0</td>
<td>191</td>
</tr>
<tr>
<td>3-5.0</td>
<td>175</td>
</tr>
<tr>
<td>5-10.0</td>
<td>92</td>
</tr>
<tr>
<td>10-15.0</td>
<td>12</td>
</tr>
<tr>
<td>15-20.0</td>
<td>2</td>
</tr>
<tr>
<td>20-25.0</td>
<td>1</td>
</tr>
<tr>
<td>25-50.0</td>
<td>4</td>
</tr>
<tr>
<td>50-100.0</td>
<td>5</td>
</tr>
<tr>
<td>100-500.0</td>
<td>12</td>
</tr>
<tr>
<td>500-1000.0</td>
<td>4</td>
</tr>
<tr>
<td>&gt;1000.000</td>
<td>4</td>
</tr>
</tbody>
</table>

Serum Cobalamin Levels and Schilling Test

These results were obtained from the patients’ charts, and radioassay tests were performed by the Radiobiology Divisions of Cincinnati’s General Hospital, Jewish Hospital, Veteran’s Administration Hospital, Good Samaritan Hospital, and Christ Hospital. Current radioassay kits in use were obtained from Diagnostic Products, Los Angeles, Calif. or Becton Dickinson, Salt Lake City, Utah.

RESULTS

Table 1 illustrates data obtained on 1118 patients who were assayed for urinary MMA. The number of patients for each range of concentration is specified. Most patients gave levels <5.0 μg MMA/ml urine. We have arbitrarily established this value as our normal range. A few patients showed slightly elevated levels between 5.0 and 20.0 μg MMA/ml urine. In most cases, the increased values can be attributed to more concentrated specimens. We have recently made creatinine measurements on urines with slightly elevated levels of MMA for a better evaluation. Nearly all patients in this study were also assayed for serum cobalamin. In cases where there was a discrepancy between serum cobalamin and urinary MMA levels, cobalamin malabsorption was tested with a Schilling test. To date, no patient with <20 μg MMA/ml has been reported by their physician to have pernicious anemia.

Table 2 lists 27 patients with clearly elevated levels of urinary MMA (>20 μg MMA/ml). Table 3 lists the patient’s neurologic abnormalities and their pertinent history. Patients 1 through 14 may have been assayed for serum cobalamin using older kits containing impure intrinsic factor. However, these patients were shown to have defective cobalamin absorption through the Schilling test or below normal serum cobalamin levels by the radioassay. The strikingly abnormal elevated levels of urinary MMA have proven to be more diagnostic of cobalamin deficiency than the serum cobalamin levels. The method of MMA quanti-
Lg MMA/ml for a normal urine. Urine and serum (ntation gave a day-to-day specimens were usually taken on the same day always prior to cobalamin therapy.

The highest urinary MMA level quantitated that was considered normal was 14 zg MMA/ml and was explained by an extremely elevated creatinine of 6.7 mg/ml. Normal creatinine levels are approximately 0.7-1.5 mg/ml urine. Creatinine measurements allow a better evaluation of urines with slightly elevated levels of urinary MMA.

Patient 4
Patient 3
Patient 2
Patient 5
Patient 1

Patient 1
Food

Patient 2
Tingling numbness for 4 wk. No motor or sensory loss. Anemia for 1 yr.

Patient 3
Numbness and tingling in hands and feet, unsteady gait 1 wk.

Patient 4
Parasthesia in hands and feet, unsteady gait 1 wk.

Patient 5
Food tastes bad, staggering gait

Patient 6
Numbness and tingling in hands and feet for 1 mo. Eighteen years previously had anemia and treated with cobalamin shots, last shot 12 yr ago.

Patient 7
Burning tongue for 6 mo. Recent myocardial infarction.

Patient 8
Inability to concentrate, poor appetite. Hair gray at age 25.

Patient 9
No neurologic abnormalities.

Patient 10
Increased feeling in lower extremities. Patient cries out in pain but cannot tell what hurts.

Patient 11
Decreased taste for food. Aching in thighs—left greater than right.

Patient 12
Palpitation, irritable.

Patient 13
Decreased memory, irritability.

Patient 14
Paralysis, slurred speech, ambulates with walker. 1.5 yr ago started having weakness in legs and difficulty in walking.

Patient 15
Speech and vision difficulty for 10 mo, trouble walking. Past diagnosis of myasthenia gravis.

Patient 16
Unable to walk without walker, weakness became worse in past year. Waddling gait with progressive weakness.

Patient 17
Food

Patient 18
Numbness and tingling in hands and feet for 6 wk. Serum folate 23.7, legs have tendency to buckle, significant underlying neurosis. Past injury.

Patient 19
Food

Patient 20
Tingling numbness for 4 wk. No motor or sensory loss. Anemia for 1 yr.

Patient 21
Trouble walking without assistance, right leg weakness greatest. Trouble walking, started 2 yr ago recurred.

Patient 22
Speech and vision difficulty for 10 mo, trouble walking. Past diagnosis of myasthenia gravis.

Patient 23
Glossodynia for 6 wk. One year previously MCV 105. Hematocrit = 32 ± 0.7.

Patient 24
Feet numb, cold and tingling of thighs. One year history of leg pain after standing.

Patient 25
Motor and sensory loss.

Patient 26
Slight tingling of toes.

Patient 27
Slight tingling of fingers and toes.

Table 3. Neurologic Abnormalities and Pertinent Past History

- Patient 1: Unable to walk without walker, weakness became worse in past year. Waddling gait with progressive weakness.
- Patient 2: Trouble walking without assistance, right leg weakness greatest. Trouble walking, started 2 yr ago recurred.
- Patient 3: Speech and vision difficulty for 10 mo, trouble walking. Past diagnosis of myasthenia gravis.
- Patient 4: Burning tongue and loss of taste for 6 mo. Recent myocardial infarction.
- Patient 5: Unable to walk without walker, weakness became worse in past year. Waddling gait with progressive weakness.
- Patient 6: Numbness and tingling in hands and feet for 1 mo. Eighteen years previously had anemia and treated with cobalamin shots, last shot 12 yr ago.
- Patient 7: Tingling numbness for 4 wk. No motor or sensory loss. Anemia for 1 yr.
- Patient 8: Palpitation, irritable.
- Patient 9: Decreased memory, irritability.
- Patient 10: Paralysis, slurred speech, ambulates with walker. 1.5 yr ago started having weakness in legs and difficulty in walking.
- Patient 11: Speech and vision difficulty for 10 mo, trouble walking. Past diagnosis of myasthenia gravis.
- Patient 12: Unable to walk without walker, weakness became worse in past year. Waddling gait with progressive weakness.
- Patient 13: Burning tongue for 6 mo. Recent myocardial infarction.
- Patient 14: Inability to concentrate, poor appetite. Hair gray at age 25.
- Patient 15: No neurologic abnormalities.
- Patient 16: Paralysis, slurred speech, ambulates with walker. 1.5 yr ago started having weakness in legs and difficulty in walking.
- Patient 17: Speech and vision difficulty for 10 mo, trouble walking. Past diagnosis of myasthenia gravis.
- Patient 18: Numbness and tingling in hands and feet for 6 wk. Serum folate 23.7, legs have tendency to buckle, significant underlying neurosis. Past injury.
- Patient 19: Food
- Patient 20: Tingling numbness for 4 wk. No motor or sensory loss. Anemia for 1 yr.
- Patient 21: Trouble walking without assistance, right leg weakness greatest. Trouble walking, started 2 yr ago recurred.
- Patient 22: Speech and vision difficulty for 10 mo, trouble walking. Past diagnosis of myasthenia gravis.
- Patient 23: Glossodynia for 6 wk. One year previously MCV 105. Hematocrit = 32 ± 0.7.
- Patient 24: Feet numb, cold and tingling of thighs. One year history of leg pain after standing.
- Patient 25: Motor and sensory loss.
- Patient 26: Slight tingling of toes.
- Patient 27: Slight tingling of fingers and toes.

DISCUSSION

Two newborns had extremely elevated levels of 2000 mg MMA/ml urine. They were diagnosed as having the inborn error methylmalonic aciduria resulting from a congenital enzyme deficiency. Another newborn had slightly elevated MMA levels. He was given 1000 mg cobalamin IM and the MMA level dropped to 4.7 g MMA/ml with normal creatines. Another newborn had a level of 15 mg MMA/ml and 3 wk later, the amount increased to 38 mg MMA/ml urine. A patient had a level of 15 mg MMA/ml urine (creatinine 0.84) and another had 13 mg MMA/ml urine (creatinine 0.7). For example, 2 kg or a Schilling test was not obtained. For example, 2 kg cobalamin deficiency, although serum cobalamin lev-
of inadvertent cobalamin injection immediately prior availability of the vitamin. Accordingly, the problem arises for MMA quantitation. The gradual reduction in urinary MMA after cobalamin therapy (Table 4) illustrates that MMA is a measure of the tissue availability of the vitamin. Accordingly, the problem of inadvertent cobalamin injection immediately prior to venipuncture (estimated 2.5%) would not negate the MMA assay as it would the serum radioassay.

The patients listed in Table 2 all showed 4 to 300 times normal levels of MMA. These elevated values have proven to be highly specific for the detection of cobalamin deficiency. Patients 2, 11, 14, and 19 may have been missed by less sensitive and specific procedures for MMA quantitation. The gradual reduction in urinary MMA after cobalamin therapy (Table 4) illustrates that MMA is a measure of the tissue availability of the vitamin. Accordingly, the problem of inadvertent cobalamin injection immediately prior to venipuncture (estimated 2.5%) would not negate the MMA assay as it would the serum radioassay.

### ACKNOWLEDGMENTS

We are indebted to Mr. Mainerd Sørensen and Mrs. Toni Kraft for technical assistance.

### REFERENCES

Cobalamin (vitamin B12) deficiency detection by urinary methylmalonic acid quantitation

EJ Norman, OJ Martelo and MD Denton