Tartrate-Resistant Acid Phosphatase in Serum of Cancer Patients

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Tartrate-resistant acid phosphatase activity determined by enzyme immunoassay was higher in the serum of cancer patients than in normal blood donors. The highest activity was found among patients having malignancy metastatic to bone. The classic colorimetric method showed a broad range of values among normal blood donors, and the contrast between normal and cancer patients was less obvious. Most of the cancer patients had normal to low alkaline phosphatase activities.

Additional Keyphrases: enzyme immunoassay and colorimetry compared · isoenzymes · alkaline phosphatase · reference interval · prostatic cancer · bone malignancy

The clinical significance of acid phosphatase for the diagnosis of prostatic cancer is well known. Extensive efforts from many laboratories have been directed toward establishing a specific analytical method for prostatic acid phosphatase. The study by Abul-Fadil and King (1) concerning inhibitor specificity of acid phosphatase among different tissues led to the recommendation of using the tartrate-sensitive fraction of acid phosphatase in serum for diagnosis of prostatic cancer (2). The clinical significance of the tartrate-resistant fraction on the other hand, is often neglected; indeed, the commercial immunochemical kits currently available have eliminated completely the measurement of the tartrate-resistant acid phosphatase.

In our experience, both tartrate-resistant and tartrate-sensitive fractions are increased in some prostatic cancer patients, although the tartrate-sensitive fraction is usually increased much more than the tartrate-resistant fraction. Our previous reports described the high activity of tartrate-resistant acid phosphatase in the serum of normal children during physiological bone growth (3, 4) and in adults with osteolytic lesions due to malignancy metastatic to bone (3–5). The increase in tartrate-resistant acid phosphatase among patients with Gaucher's disease (6–8) and dengue fever (9) is also dependent on bone involvement.

In evaluating an enzyme immunoassay procedure (10) we developed recently for the analysis of acid phosphatase band 5 in serum, we have studied the variation of tartrate-resistant acid phosphatase among cancer patients. The data obtained were compared with those by a colorimetric method involving p-nitrophenyl phosphate as substrate. We also compared our results with the patients' activities of alkaline phosphatase, which is well-known to be related to osteoblastic activity (11).

Materials and Methods

The colorimetric method and enzyme immunoassay procedure were described previously (10). Alkaline phosphatase was determined with reagent kits supplied by Sigma Chemical Co., St. Louis, MO.

Serum specimens were stored at ~70 °C. Serum from 32 normal adult blood donors was supplied to us by the American Red Cross Blood Service, Northeastern New York Region. Samples from 134 cancer patients were obtained from the Oncology Clinics of the Columbus Hospital Regional Oncology Center, Great Falls, MT. Most commonly, the patients had primary tumors located in the prostate and breast; next most common were carcinomas of the lung and colon-rectal area, followed by small numbers of cases of many other primary tumors. Multiple samples were obtained on all patients with malignancy, for a total of 479 specimens. The data shown in the Figures are the average values for each patient.

All diagnoses of malignancy had been histologically confirmed. Metastatic disease was evaluated by observation during surgery, results of standard automated determinations of blood analytes, and radiographic studies including ordinary roentgenograms and radionuclide scans of bone, liver, and brain, as well as computerized axial tomographic scanning.

The blood samples from cancer patients were collected with blood for other routine blood tests. After clotting and centrifugation, the serum was removed and stored at ~70 °C until analysis.

The samples were divided into four groups: group 1, from normal adult blood donors; group 2, from cancer patients without known metastases; group 3, from patients with cancer cells metastatic to lymph nodes or other tissues without involvement of the bone; and group 4, from patients with cancer cells metastatic to bone. Prostatic cancer patients were distributed between groups 2 and 4, there being no prostatic cancer patient with metastatic disease to soft tissues without bone involvement (group 3).

Results

The activity of tartrate-resistant acid phosphatase as determined by the enzyme immunoassay (Table 1) was increased in patients with malignancy, with and without metastatic disease. The highest values were observed in the patients with metastases to bone (group 4), but tartrate-resistant acid phosphatase was also increased in patients with metastatic disease to nodes and other tissues than in patients with no known metastases. Eighty-five percent of group 4 had values above the normal range, compared with 44% of group 3 (Figure 1).

As determined by the colorimetric method, activities of tartrate-resistant acid phosphatase were similar among groups 1, 2, and 3, but was increased in group 4 (Table 1). As Figure 1 shows, most of the data in groups 2, 3, and 4 were within the normal range, with only 30% of the values for group 4 outside the normal range.

Most of the cancer patients had low alkaline phosphatase activity in their serum. Several samples in groups 2 and 4 had below-normal alkaline phosphatase activity (Table 2,
Table 1. Tartrate-Resistant Acid Phosphatase Activity (U/L, Mean ± SE) Determined by Enzyme Immunoassay (EIA) and Colorimetry (Col)

<table>
<thead>
<tr>
<th>Site of cancer</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EIA</td>
<td>Col</td>
<td>EIA</td>
</tr>
<tr>
<td>Prostatic</td>
<td>n</td>
<td>±</td>
<td>n</td>
</tr>
<tr>
<td>Breast</td>
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<td>7.8 ± 0.8</td>
<td>14.1 ± 1.5</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>6.6 ± 2.5</td>
<td>11.4/2.4</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>6.4 ± 0.4</td>
<td>11.0 ± 3.1</td>
</tr>
</tbody>
</table>

Normal values (group 1): EIA, 3.5 ± 0.3; Col, 10.4 ± 0.5 (n = 32 each).

* n = 32.

discussion

The present data confirmed previous observations of high activities of tartrate-resistant acid phosphatase among cancer patients with cancer cells metastasized to bone, and showed that the immunochemical assay indicates this more satisfactorily than the colorimetric method. The enzyme immunoassay produced lower values of tartrate-resistant acid phosphatase in serum of normal adults and higher values in cancer patients than did the colorimetric method; as a result, the difference between the two groups of subjects is more obvious, presumably because of the higher specificity of the enzyme immunoassay method. The major tartrate-resistant acid phosphatase in serum is osteoclastic acid phosphatase band 5b and erythrocytic phosphatase. The proportion of the latter is quite variable, depending on the extent of hemolysis of the sample. Precipitation of band 5b from the serum by a specific antibody, as in the enzyme immunoassay, eliminated the variable contributions from erythrocytic acid phosphatase so that the values for tartrate-resistant acid phosphatase in normal adults were lower and fell within a narrower range than the data obtained by the colorimetric method.

Among cancer patients, on the other hand, the tartrate-resistant acid phosphatase released from osteoclasts may be partially inactivated during storage. Our previous data (10) showed that inactivated purified acid phosphatase band 5b was reactivated by precipitation of the enzyme with the antibody. Therefore, the serum from cancer patients gave higher values by the enzyme immunoassay than by the colorimetric method. We would thus expect the enzyme immunoassay to give lower values for normal serum, where contamination by erythrocytic phosphatase is relatively high compared with the amount of isoenzyme band 5, and higher values in cancer patients, where erythrocytic phosphatase contamination is less than the amount of inactivated osteoclastic phosphatase.

Some of the "nonmetastatic" cancer patients (group 2) could have a small number of cancer cells in bone, which would be difficult to detect by bone scan. Among different types of cancer, bone involvement is seen most often among breast cancer and prostatic cancer, and the increase in tartrate-resistant acid phosphatase in the group 2 samples was, in fact, higher among patients with breast cancer or prostatic cancer than other cancer patients.
All of the cancer patients were being treated at the time of sampling, but the exact clinical status of each was not available. The low activity of tartrate-resistant acid phosphatase in some patients may have been due to a good response to treatment. We will consider the tartrate-resistant acid phosphatase activity of individual patients at different stages of treatment in future studies.

Alkaline phosphatase is an enzyme marker of osteoblastic activity (11, 12); most of the cancer patients in this study, however, did not have increased serum activities of alkaline phosphatase. The few instances of extremely high activities of alkaline phosphatase, but normal tartrate-resistant acid phosphatase, could be due to damage of tissues other than bone.

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References


Some Factors Affecting Determination of Carotenoids in Serum

Micheline M. Mathews-Roth and Meir J. Stampfer

Because of increasing interest in carotenoid pigments, we conducted a study of the methods of determining carotenoids in serum. We found 1 mol of KOH per liter of absolute methanol to be the most effective saponifying solution. The absorbance of beta-carotene in petroleum ether, the extraction solvent, is proportional to dilution up to an absorbance of 0.85 at 450 nm. Beta-carotene in petroleum ether solution is not impractically sensitive to ambient light at room temperature. However, if vitamin A is also to be measured in these serum specimens or petroleum ether extracts, exposure to light should be minimized. We found that serum may be shipped either in cold packs or at ambient temperature (tested up to five days) without significant change in carotenoid concentration. Serum samples for carotenoid determination are best stored at −70 °C: samples stored at −20 °C deteriorate substantially over several months.

Additional Keyphrases: sample handling • variation, source of

Beta-carotene is prescribed to ameliorate the photosensitivity associated with erythropoietic protoporphyria and other light-sensitive diseases (1). Recently, much interest has been generated by reports that beta-carotene or vitamin A may offer protection against the development of some forms of cancer (2). As a result, more laboratories probably will be measuring carotenoids in serum. We therefore closely examined the traditional method of determining serum carotenoids, as well as the effect of different specimen-handling procedures on carotenoid concentrations.

Materials and Methods

Apparatus. We used a Cary Model 14 recording spectrophotometer (Varian Instruments, Palo Alto, CA) in determining absorption spectra of carotenoid-containing solutions. Specimens were shaken during the extraction procedure on a Kraft Model 5-500 "Shaker in the Round" fitted with a Model RD-20 head and three Model SR 815 centrifuge tube racks, which can be turned while attached to the shaker so that the tubes can be shaken with their length parallel to the table top, thus affording efficient extraction (Kraft Instrument Co., Mineola, NY). In this apparatus, 24 tubes can be shaken at one time.

Reagents. Two reagents are needed for the extraction of carotenoids from serum. For saponification, a 1 mol/L solution of KOH in absolute methanol is used (dissolve 56 g of KOH in ACS Certified reagent-grade methanol and dilute to 1 L), and for extraction of the saponified carotenoids, petroleum ether (boiling range 35–60 °C, ACS Certified reagent).

Carotenoid extraction procedure. We have used the method described here since 1969 in our studies of carotenoid treatment of photosensitivity in various diseases. In the present study, however, blood was obtained from normal