Clinical Applicability of Acid Phosphatase Isoenzyme Assay

Tsieh Sun, Kwok-Wal Lam, and Leela Narukar

We compared electrophoretic evaluation of acid phosphatase isoenzymes with spectrophotometric determination of prostatic acid phosphatase in terms of clinical utility. In all of 33 cases of prostatic carcinoma, an increased prostatic fraction was detected; in nine prostatotomized patients, this fraction returned to normal as measured by either technique. Abnormal spectrophotometric results were also seen in 10 cases of benign prostatic hypertrophy and seven cases of non-prostatic disorders, but only two benign prostatic hypertrophy and one non-prostatic case showed a prostatic band (band 2) in an electrophoretogram. Band 2 was not demonstrated in 463 patients affected by a great variety of diseases but without prostatic disorders. A weak band 5 was seen in patterns for most patients, except for cases with metastatic bone tumor and Gaucher's disease, whose serum showed a strong band 5. The specificity of bands 2 and 5 seems to be confirmed by this large series of patients. Measurement of acid phosphatase isoenzymes is recommended as a routine screening test for patients whose serum acid phosphatase is abnormally high, because the isoenzyme study not only indicates the presence or absence of prostatic cancer but also whether or not there is bony metastasis. Other disorders such as Gaucher's disease, different kinds of leukemias, and thrombocytopenia may also be detected and distinguished by this screening technique.

Additional Keyphrases: isoenzymes, cancer, prostatic tissue, Gaucher's disease, screening, spectrophotometry, electrophoresis on acrylamide gel, "Kli" methods, thrombocytopenia.

For many years, enzyme assays have played an important role in the diagnosis of different diseases. Isoenzyme evaluations further help in localizing tissue or organ damage. However, routine assays of isoenzymes in clinical laboratories have been limited to lactate dehydrogenase and creatine kinase. The value of acid phosphatase (EC 3.1.3.2) isoenzyme analysis, though well proven by research (1-4), is not commonly recognized by clinical laboratories. Current efforts seem to be concentrated on only a single fraction, isoenzyme 2 of prostatic origin (5-8).

However, as a screening tool for the differential diagnosis of diseases that involve increased serum acid phosphatase, electrophoresis on acidic acrylamide gel is the method of choice. The major drawback of the isoenzyme approach is the time-consuming procedure in preparing the three layers of acidic acrylamide gel in the columns. A recently marketed acrylamide gel kit stimulated our interest in evaluating the usefulness of this test in general and the diagnostic value for prostatic cancer in particular. A comparison of the electrophoretic and spectrophotometric analyses is reported.

Materials and Methods

Patients

This study included 500 patients (357 male, 143 female). The patients' ages ranged from neonate to 95-year-old; most were older than 16 years. Their diseases covered a wide variety, including coronary heart disease, uterine fibroid, diabetes, duodenal ulcer, hepatic cirrhosis, inguinal hernia, nephrotic syndrome, renal failure, hepatitis, splenic anemia, head trauma, gastrointestinal, diverticulitis, rheumatomyosarcoma, pancreatic carcinoma, otitis media, metastatic bone tumor, hypertension, breast carcinoma, intestinal obstruction, emphyma, Gaucher's disease, urethral stenosis, pharyngitis, hyperlipidemia, lung cancer, organic brain syndrome, appendicitis, hydrome, normal pregnancy, prostatic carcinoma, and benign prostatic hypertrophy. Spleen specimens from hairy cell leukemia, leukocytes from cases of chronic lymphocytic leukemia, and seminal fluid were used as references to identify the location of bands 2, 3, and 5. The major isoenzymes of the prostate, leukocytes, and hairy cells are bands 2, 3, and 5b, respectively (5). We reviewed the medical records of all patients, looking especially for conditions that could cause increased acid phosphatase, such as prostatic or bone diseases.

Spectrophotometry of acid phosphatase. We used the spectrophotometry with p-nitrophenyl phosphate as substrate for routine assays of acid phosphatase in serum (9). Serum specimens containing <15 U of acid phosphatase per liter were considered normal.

Electrophoresis on acidic acrylamide gel. Acid phosphatase isoenzymes were separated on acidic acrylamide gel column as previously described (10). The activity bands were made visible by staining the gel column with 1-naphthyl phosphate Fast Garnet GBS for 1 h. The acrylamide gel columns, electrophoresis buffer, and staining solution were supplied by Eureka Laboratories, Inc., Sacramento, CA 95816. The intensity of the bands was recorded with a Gilford spectrophotometer equipped with a linear transport. The band intensity was compared with that of a known activity (0.2-2 mU) of isoenzyme 5b, isolated from a spleen affected by leukemic reticuloendotheliosis (17).

Results

Most (460) of the 500 cases showed normal acid phosphatase activity by spectrophotometry (Table 1). Of the 50 cases showing abnormally high prostatic acid phosphatase in their serum, as detected by the spectrophotometric method, 33 were prostatic carcinoma of stages C and D; 10 were benign prostatic hypertrophy, and seven had no clinical symptoms of prostatic disorders. The patients with normal acid phosphatase values included nine with prostatic cancer (prostatetomized) and 53 with benign prostatic hypertrophy.

Analyses for acid phosphatase isoenzyme showed band 2 in all of the 33 cases of prostatic carcinoma before surgical treatment (Figure 1). Band 2 was also seen in two cases of benign prostatic hypertrophy and one case of urethral stricture. Band 2 was absent from the patterns for nine prostatic-carcinoma patients after surgical removal of the tumor.

Band 5 was present in most samples (434 cases). Its inten-

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Table 1. Spectrophotometric and Electrophoretic Analyses Compared for Detection of Prostatic Acid Phosphatase

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Spectrophotometry</th>
<th>Electrophoretic assay of band 2</th>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Prostatic carcinoma</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>Present</td>
</tr>
<tr>
<td>Prostatic carcinoma,</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>postoperative</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Benign prostatic</td>
<td>53</td>
<td>10</td>
</tr>
<tr>
<td>hypertrophy</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>Non-prostatic disorders</td>
<td>388</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>450</td>
<td>50</td>
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</table>

As the spectrophotometric technique. Although other methods such as radioimmunoassay (6) and counterimmunoelectrophoresis (8) are more sensitive than electrophoresis for detecting early prostatic cancer, they are not necessarily more specific. For instance, cross reactivity to antibody against prostatic fraction has been reported in one case of pancreatic carcinoma (12) and one case of chronic granulocytic leukemia (13) by immunochemical techniques. Because pancreas and granulocytes both contain band 2 isoenzyme as does the prostate (3), cross reactivity is not unexpected. In this study, band 2 was not seen in the pattern for a patient with pancreatic carcinoma, but was demonstrated in a case of urethral stricture, perhaps as a result of seminal fluid leaking into the blood stream. The absence of band 2 in only one case of pancreatic carcinoma, however, does not imply a higher specificity of the electrophoretic technique.

In situations where the source of supranormal acid phosphatase is unknown, an electrophoretic analysis apparently is helpful as a screening technique as well as a means for monitoring treatment. This study showed the absence of band 2 after prostatectomy and the appearance of a strong band 5 when prostatic carcinoma metastasizes to bone (14). We believe that a series of isoenzyme determinations would provide a kinetic picture of the clinical situation—something not feasible from data on analysis of a single fraction. Although a strong band 5 is also present in patterns for samples from Gaucher's disease, this is readily distinguished clinically from metastatic cancer (15).

Band 3 was only seen in 16 of the 500 cases we examined. In a normal serum specimen, this represents the release of this isoenzyme from platelets during blood clotting (3). Markedly increased, it may indicate diseases in which platelet or lymphocyte counts are abnormally high, such as thrombocytopenia or lymphocytic leukemia (1, 2). Our only case of lymphocytic leukemia supports this idea. A great potential of acid phosphatase isoenzyme analysis lies in the distinction of different leukemias, because monocytes and myelocytes contain band 2 and band 4 isoenzymes, which differ from the band 3 isoenzyme seen in lymphocytes (1, 2).

The electrophoretic pattern obtained with the commercial electrophoresis kit is identical to the results previously described by Lam et al. (10). In several occasions, there were variations of the electrophoretic mobility of band 5, which might result from some unknown factors in the serum. The variations were minimized by doubling the volume of the sample buffer. A mixture of 0.05 mL serum with 0.2 mL of sample buffer gave highly reproducible results.

The apparatus supplied by Miles Laboratory allows simultaneous analyses of 36 samples. The entire procedure involves: (a) placing the samples on top of the gel columns and attaching the gel columns to the electrophoresis apparatus and (b) removing the gel columns from the glass tube and placing them in the staining solution. The actual working time for 36 samples include 30 min in step a and 30 min in step b. It is about the same time required for radioimmunoassay, and about twice as long as that for spectrophotometric analysis. Therefore, qualitative analysis of acid phosphatase isoenzymes with the use of the commercial kit will eliminate about 3 h of work in preparation of gel columns and thus make it feasible for use in a busy clinical laboratory. An additional 5 min per sample is required for densitometric quantitation of the staining intensity of individual bands.

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References

Multicomponent Radioimmunoassay: Simultaneous Measurement of Choriomammotropin and Pregnancy-Specific \( \beta_1 \)-Glycoprotein in Pregnancy Serum

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We describe a receptacle\(^1\) for use in the simultaneous radioimmunoassay of two serum constituents in a single sample, and the application of this principle for the measurement of human pregnancy serum choriomammotropin and pregnancy-specific \( \beta_1 \)-glycoprotein. The analytical performance of this multicomponent radioimmunoassay approaches that of conventional radioimmunoaassays. By following the principle described, it is likely that large numbers of constituents can be efficiently measured in single samples.

The rapidly increasing number of radioimmunoassays has led to the development of automated techniques to increase the efficiency of laboratories performing these assays (1–3). Major steps in the advancement of instrumentation have included development of efficient sample processors and multi-detector counting devices with appropriate data-processing facilities. In parallel with the increased efficiency in sample handling and radioactivity measurement, it would be desirable to develop the radioimmunoassay itself to allow multi-component assays in single specimens. Here we describe such a principle. It is based on the use of immobilized antibodies (4) against the constituents to be measured, fixed to the surface areas of a reaction vessel, which can be detached for separate coating and counting. As a practical application, we describe a technique for the simultaneous measurement of human pregnancy serum choriomammotropin (hPL) and pregnancy-specific \( \beta_1 \)-glycoprotein (SP\(_1\)). The antibodies against these two proteins are separately immobilized on the inner surfaces of two separable parts of a test tube, which serves as the reaction vessel for two radioimmunoassays from a single sample.

Materials and Methods

Reagents

SP\(_1\) was purified from human placentas by a modified immunoadsorption method (Koskinen et al., to be published) based on that by Bohn et al. (5). \([^{125}\text{I}]\text{SP}_1\) was prepared according to Hunter and Greenwood (6) with use of \(\text{Na}^{125}\text{I}\) purchased from the Radiochemical Centre, Amersham, U.K. HP7 9LL. Anti-SP\(_1\)-antibody was supplied by Dako Immunoglobulins, Copenhagen, Denmark. hPL standards, \([^{125}\text{I}]\text{hPL}\), and anti-hPL-antiserum were kindly donated by Nordiclab, Oulunsalo, Finland. Bovine serum albumin was from Armour Pharmaceutical Co. Ltd., Eastbourne, U.K., and other chemicals were from Merck AG, Darmstadt, F.R.G.

Multi-RIA-tubes

Figure 1 shows the structure of the tubes used. For the purpose of this study, the lower part (volume, 1 mL) was cut from a polystyrene tube (NUNC Products, Roskilde, Denmark), 11 mm o.d., and the upper cylindrical part was cut from...