CHAPTER TWO

Acid Phosphatase in Cancer.
INTRODUCTION

During past four decades we have witnessed significant developments in the clinical application and interpretation of both serum alkaline and acid phosphatases. Under the name of acid phosphatase are included all phosphatases with optimal activity below pH of 7.0. The greatest concentrations of acid phosphatase activity are present in liver, spleen, milk erythrocytes, platelets, bone marrow, and the prostate gland. The prostate is the richest source, and it contributes about one-third to one half of the enzyme present in serum of healthy males.

The serum prostate levels are a useful tool in the diagnosis and management of prostatic diseases. It is now apparent that acid phosphatases (ACP) represent a heterogeneous group of enzymes containing many isoenzymes, each specific for one type of tissue. The present trend in study of the acid phosphatases includes the combined use of biochemical, electrophoretic, electron microscopic and immunologic techniques to further understanding
of the relationship between these isoenzymes
and the cellular and subcellular fractions of
various tissues.

Acid phosphatase activity is heterogeneous
in various tissues (1-2). Some of these ACP
are organ specific, cell specific, and possibly
subcellular organelle specific (3-8). It is
redundant to say this enzyme as extremely important
in the diagnosis of metastasizing carcinoma of
the prostate. Gutman (9) reported that ACP is
elevated to several hundred fold in subjects with
prostatic carcinoma, especially when the tumor is
extended outside the gland, when compared with the
normal subjects. Fishman et al. (10-11) reported
a method for "prostatic" acid phosphatase which
gave an excellent correlation with the proven cancer
of the prostate. They also reported that by means
of an l-tartarate inhibitor, one could measure
largely, but not exclusively prostatic acid
phosphatases based on both experimental and clinical
data. Reynolds et al. (12) used copper to inhibit
erythrocyte ACP and reported a large number of
patients with widespread cancers of the female breast or of the prostate gland having significantly elevated values for copper resistant ACP.

Abdul-Fadl and King (13) employed a simple formaldehyde treatment to distinguish between the high ACP of prostatic origin and those accompanying other conditions. Gray (14) reported that the plasma acid phosphatase derived from the prostate was inactivated by treatment with alcohol and this behaviour was useful in deciding whether an increased ACP activity level was due to prostatic carcinoma or to liberations of the ACP from red blood cells by hemolysis. Meijer (15) reported three different non-specific fractions characterized by different pH optima between 3.4 - 5.8 in the liver and spleen. Hudson et al. (16) demonstrated that liver appeared to be implicated in the metabolism of serum ACP of prostate origin and found values grossly elevated in cases with metastases of the liver from prostatic cancer. Ladehoff and Rasmussen stated that the increased fibrolysis in blood observed in transvesical prostatectomy was caused by a release of enucleation
together with damage of adsorption from the particularly active "surgical capsule" which was the site of cleavage. Adventitious elevations of ACP have appeared frequently in the literature. It has been noted that massage, palpation, trauma, rectal examination or any pressure exerted on prostate may result in sudden elevation of the ACP activity level.

Huggins and Hodges (18) published their classic paper on acid phosphatase and prostate carcinoma. They reported elevated levels of ACP in prostate cancer and bone metastases. Serum ACP was found to be increased in 19 of 25 patients with prostatic carcinoma and bone metastases. The elevation of serum ACP in carcinoma of prostate without metastases may be conceived as due to either to a mechanical block to the drainage of ACP into the urethral sinus or else to some other inability of the epithelial cells in the glands to secrete the enzyme through the ducts into the sinus. Consequently the enzyme is refluxed back through the blood vessels and possibly lymphatics into the circulation. In ..
human neoplasms enzyme ACP activity is strongest in prostate, variable in breast, stomach, and colon, and weak in thyroid, kidney and ovary (19). It has been reported by large number of workers that serum ACP was increased in patients with prostatic carcinoma (20-21). Misseran (22) postulated by employing histochemical and quantitative microchemical methods that carcinoma of the breast, bronchus, skin, bladder, and gastrointestinal tract were richer in ACP than the tissue of origin. Similar results have been reported by Gomori (23) with histochemical studies on human tumors.

Lemon, Reynolds, and Kelley (24) claimed that ACP levels were elevated to 74% in a group of female patients with mammary metastatic carcinoma(9). Similarly, Koudstaal et al. (25) reported elevated levels of serum acid phosphatase in mammary carcinomas. Machinami (26) demonstrated using histochemical techniques, that total ACP activity is increased in malignant mammary tumor tissue with respect to adjacent non tumor tissue and benign tumor tissue. Filmus et al. (27) demonstrated
that total ACP activity in malignant breast
tumors was elevated than normal breast tissues,
whereas the benign tumors had intermediate
activity. Jegathesan et al. (28) and Tavassoli (29)
reported increased levels of total serum ACP in a
significant number of patients with breast cancers
and bone metastasis.

Taking into consideration the fluctuating
results of various workers, the present work was
planned to formulate a comparative data of acid
phosphatase in different cancers with respect to
different age groups.

**MATERIAL AND METHODS**

Sera included in this study was obtained from
Patients admitted in the Medical College and Hospital,
Aurangabad. Before carrying out the enzyme estimation,
it was ascertained that the patient had clinical and
radiological evidence of malignancies. Details of
the number and type of cancer studied are given in the
table - 2. The patients were divided into two major
groups viz., rural and urban. These were further
divided into 3 age groups 21 - 40 years and 41 - 60 years
and 61 - 80 years. The cancers studied for these age groups were the cervical, the ovarian, and the breast carcinomas. The female subjects were in their III stage of the disease. For the colon, stomach, and the rectal carcinomas the cancer subjects were divided according to their sexes, for both the habitats.

Determinations were also made in sera from 140 individual without malignancies for other causes of serum and acid phosphatase elevation. Many of these sera were from healthy employees of the Medical College and also from healthy people undergoing routine checkups. These subjects ranged in age from 20 to 80 years.

Sample collection was done as described under alkaline phosphatase.

Enzyme assay

The enzyme assay was carried out by the method of Gutman and Gutman (39). The enzyme activity was determined by estimating the amount of phenol liberated at 37°C under defined conditions. The reaction
was carried out at 37°C for 60 minutes in a total volume containing 2 ml of citrate buffer 0.2 M (pH 4.9), 1.0 ml of substrate di-sodium phenyl phosphate (0.02 M) and 0.2 ml of serum. The reaction was terminated by addition of 1.8 ml of Folin-Ciocalteau reagent and 1.0 ml of sodium carbonate (20%). The colour complex was measured on Erma colorimeter at 660 nm after 30 minutes using phenol as standard. The results are presented in international units per litre of serum (U/L).

**RESULTS**

Elevated levels of serum acid phosphatase were noted down in all the cancers studied. Increase in the enzyme activity was variable in different carcinomas (Table 1). It was highest in the breast carcinoma.

Serum acid phosphatase activity was significantly higher in cervical cancer than normal. The percentage induction was greatest in the age group of 21 – 40 years. The magnitude of induction in the three age groups of rurals was 113%, 33% and 64% respectively, and in the urbans it was 125%, 113% and 62% respectively.
<table>
<thead>
<tr>
<th>Type</th>
<th>Age</th>
<th>Rural</th>
<th>n</th>
<th>Urban</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>21 - 40</td>
<td>4.73 ± 1.2</td>
<td>15</td>
<td>5.6 ± 1.2</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>5.98 ± 1.8</td>
<td>20</td>
<td>5.4 ± 1.0</td>
<td>35</td>
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<tr>
<td></td>
<td>61 - 80</td>
<td>7.30 ± 2.0</td>
<td>15</td>
<td>6.2 ± 1.5</td>
<td>25</td>
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<td>Cervical</td>
<td>21 - 40</td>
<td>B 10.10 ± 1.6</td>
<td>30</td>
<td>B 12.63 ± 2.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 6.80 ± 1.8</td>
<td></td>
<td>A 7.60 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>B 7.98 ± 1.2</td>
<td>35</td>
<td>B 12.60 ± 3.2+</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 5.20 ± 1.0</td>
<td></td>
<td>A 6.86 ± 1.4</td>
<td></td>
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<tr>
<td></td>
<td>61 - 80</td>
<td>B 11.97 ± 1.5</td>
<td>20</td>
<td>B 10.10 ± 2.0</td>
<td>25</td>
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<tr>
<td></td>
<td></td>
<td>A 9.65 ± 1.2</td>
<td></td>
<td>A 5.89 ± 1.0</td>
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<td>Ovarian</td>
<td>21 - 40</td>
<td>B 10.10 ± 2.0</td>
<td>20</td>
<td>B 12.95 ± 3.3+</td>
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<td></td>
<td></td>
<td>A 6.2 ± 1.2</td>
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<td>A 12.60 ± 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>B 21.28 ± 4.2+</td>
<td>30</td>
<td>B 12.39 ± 3.0+</td>
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<td></td>
<td></td>
<td>A 15.16 ± 1.9</td>
<td></td>
<td>A 14.66 ± 2.4</td>
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<tr>
<td>Breast</td>
<td>21 - 40</td>
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<td>25</td>
<td>B 21.00 ± 2.8+</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 15.80 ± 3.0+</td>
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<td>A 14.60 ± 2.9+</td>
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<td></td>
<td>41 - 60</td>
<td>B 21.50 ± 3.6+</td>
<td>35</td>
<td>B 19.95 ± 2.0</td>
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<td></td>
<td></td>
<td>A 16.20 ± 2.8+</td>
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<td>A 12.80 ± 2.0</td>
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<td>61 - 80</td>
<td>B 35.90 ± 3.0+</td>
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<td>B 31.92 ± 4.0+</td>
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<td>A 24.04 ± 2.8+</td>
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<td>A 20.89 ± 3.0+</td>
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B - Before treatment  A - After treatment  + p < 0.05  {27}

n - in parentheses indicate number of subjects.
### Table

**Serum Acid phosphatase in colorectal cancer**

<table>
<thead>
<tr>
<th>Type</th>
<th>Rural</th>
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<th></th>
<th>Urban</th>
<th></th>
<th></th>
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<td></td>
<td>Male</td>
<td>Female</td>
<td>n</td>
<td>Male</td>
<td>Female</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>6.0 ± 1.2</td>
<td>6.2 ± 1.2</td>
<td>35</td>
<td>5.7 ± 1.5</td>
<td>6.0 ± 6.4</td>
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<tr>
<td>Colon</td>
<td>B 10.64 ± 2.0</td>
<td>7.98 ± 1.3</td>
<td>17</td>
<td>B 9.31 ± 2.0</td>
<td>11.97 ± 1.8</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>A 8.90 ± 1.5</td>
<td>5.60 ± 1.0</td>
<td>17</td>
<td>A 7.00 ± 1.0</td>
<td>6.00 ± 2.0</td>
<td>16</td>
</tr>
<tr>
<td>Stomach</td>
<td>B 14.63 ± 3.0⁺</td>
<td>11.97 ± 2.0</td>
<td>14</td>
<td>B 17.29 ± 3.0⁺</td>
<td>10.64 ± 1.6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>A 10.60 ± 2.0</td>
<td>8.60 ± 1.6</td>
<td>14</td>
<td>A 10.86 ± 2.8⁺</td>
<td>5.56 ± 1.8</td>
<td>15</td>
</tr>
<tr>
<td>Rectal</td>
<td>9.31 ± 1.6</td>
<td>6.65 ± 1.2</td>
<td>15</td>
<td>10.64 ± 2.0</td>
<td>7.98 ± 1.9</td>
<td>15</td>
</tr>
</tbody>
</table>

n = in parentheses indicate number of subjects

B = Before treatment
A = After treatment

**P < 0.05**
In the ovarian carcinomas a similar trend was observed as in cervical cancers. The magnitude of increase of serum ACP was found to be highest in the age group of 41 - 60 years for both the habitats. In the rurals the percent induction for the two age groups was 113 and 256 respectively. In the urbans percent induction was 256% and 340% respectively.

Patients with breast cancers exhibited the highest elevations of total serum ACP activity. The magnitude of induction for the rural age group observed was 349%, 259% and 391% respectively. For the urbans the percent induction noted was 344, 267 and 415 respectively.

In the carcinomas of colon, stomach, and rectal the ACP activity was found to be more in the males than the females for both the rural and urban habitats.

**DISCUSSION**

Measurement of total serum ACP demonstrated that malignant mammary tumors had greater activity than the normal subjects. The activity was greatest
in the older subjects of the age group of 61 - 80 years. The elevation of the enzyme correlates with the cancer activity. Cancerous breast tissue contains greater ACP than the normal tissue. The primary tumor may not liberate sufficient acid phosphatase to raise the serum enzyme levels. Raised serum ACP is predominant, if not always, due to the presence of metastasis.

Muscle wasting is a probable cause of elevated serum glycolytic enzymes in the cancer which would appear unlikely to be an important factor causing elevation of serum ACP. Similar results in breast carcinomas have been reported by earlier workers (11, 16, 17, 31).

In all the other cancers studied the activity of the enzyme was elevated in comparison with the normals. In cervical and ovarian carcinomas the elevation indicates the spread of the neoplasm, even though the other evidences of metastasis are lacking. The elevation of the enzyme occurs only when the tumors has spread to lymph nodes or bone marrow spaces.
The results of this study indicate that the elevation of serum acid phosphatase may occur in the course of different non-prostatic cancer and that, moreover, its occurrence is associated with bone metastasis, demonstrable by radiologic and radioactive scanning. Determination of serum acid phosphatase activities in cancer patients may, therefore, offer a less expensive and less hazardous alternative to radiologic and radioactive scanning methods for the detection of bone metastasis. Serum ACP is also a useful and convenient additional parameter in the assessment of breast cancer activity along with other enzymes of similar origin. Secondly, nutritional factors may play an important role as high fat diet has led to more tumor incidences and number. In mammary tumors the promoting effects of high fat diet may be due to triglycerides.
REFERENCES

1. Herbert, F.K., Quert, J., Med., 59: 221 (1942)


