Chapter Four-

ARGINASE IN CANCER
INTRODUCTION

Arginase was first described by Kossel and Dakin (1). It is an enzyme of urea cycle having absolute specificity for L-arginine which it hydrolysis to L-ornithine and urea.

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 & \quad \text{NH}_2 \\
\text{C}=\text{NH} & \quad (\text{CH}_2)_3 \quad \text{CO} \\
\text{NH} & \quad \text{NH}_2 \\
(\text{CH}_2)_3 + \text{H}_2\text{O} & \rightleftharpoons \text{CH(NH}_2) + \text{UREA} \\
\text{CH (NH}_2) & \quad \text{COOH} \\
\text{COOH} & \quad \text{ORNITHINE}
\end{align*}
\]

ARGININE

It is present mainly in the liver predominantly in the cell nuclei and microsomes. Much lower levels of arginase activity are found in other organs such as the kidney. The level of activity in the liver is responsive to nutritional and hormonal influences (2-4). The arginase activity of normal human serum is very low, that of erythrocytes is 200 times greater. Lymphocytes
and blast cells of all types have little or no arginase activity, but neutrophilic leucocytes possess 50 times the activity of red cells (5-7).

High levels of arginase were found in patients with hepatoma, infective hepatitis and fatty infiltration of the liver, but normal levels were observed in a patient in hepatic coma (8).

Raised serum arginase activities are also encountered in children with typhoid fever. In these patients the serum arginase parallels the serum transaminase, reaching a maximum in the second and third weeks and returning to normal after six or seven weeks (9). Elevated levels of arginase were also reported in patients with pernicious anaemia and other nutritional macrocytic anaemias than the healthy subjects. The enzyme level returned to normal when the patient responded to treatment, but relapse was accompanied by increased enzyme activities. Elevated arginase activities were also found in thalassaemia major, but in thalassaemia minor the erythrocytes of most
patients displayed activities which were either in or only slightly exceeding the normal range.

It has been proposed that arginase isoenzymes play a role in argininaemia, a disease characterized by arginase deficiency (10). No significant differences were detected between the leucocyte arginase activities of normal subjects and in patients with haematological diseases (11).

Arginase has been purified from human blood serum and it has been observed that the specific activity of the purified enzyme was 2400 fold greater than that of crude serum. In this content, it was thought worthwhile to investigate the changes in serum arginase with various carcinomas.

MATERIAL AND METHODS

Male and female subjects between the age group of 20 years to 80 years, having no significant diseases were considered as healthy controls. The normal subject comprised of 70 rurals and 85 urbans. The cancer group was composed of male and female
subjects who were the inpatients of Medical College and Hospital, Aurangabad. These patients were diagnosed as carcinomatous depending upon the clinical examination, which was further confirmed by histopathological studies of the tissue.

The diseased subjects were divided into two main groups depending upon their habitat viz., rural and urban.

Sample collection:

As described in earlier chapter (Chapter-3).

Assay

The method followed for arginase estimation was of Abdus Salam (12) in which ornithine was used as a standard. In this assay ornithine formed from the substrate arginine, was reacted with ninhydrin to form a coloured complex. 0.5 ml of serum was added to both tubes. Tubes were incubated for 5 minutes and then 1.0 ml of buffered substrate, (L-arginine HCl 0.53%) was added to the 'T' tube. After incubation for 20 minutes at 37°C, 4 ml of ninhydrin (0.75%) was added to both the tubes and 1.0 ml of buffered substrate was added
to the 'B' tubes. Tubes were incubated at 95° for 15 minutes. After cooling, the colour developed was measured at 530 nm.

The arginase activity was measured in m U/L.

**RESULTS**

The urban normals indicated greater arginase activity than the ruralas. Table 1 indicates the serum arginase activity in the normal and female cancers i.e. cervical, ovarian and breast cancers. A slight elevation in the serum enzyme activity was noted. The magnitude of induction was more in the urban subjects than ruralas, especially in the age group 21 - 40 years. The magnitude of elevation was 18.5%, 15.4%, and 7.6% in ruralas and that in urbans 3.5%, 11% and 10% respectively.

Serum arginase activity was found to be elevated in ovarian tumors than normals. The percent induction was 78% and 67% in ruralas and 27% and 64% in urbans.

Elevated levels of the enzyme were noted in breast cancers, the increase was more in the
<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>7.0 ± 1.2</td>
<td>20</td>
<td>8.5 ± 1.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>41-60</td>
<td>6.2 ± 1.0</td>
<td>25</td>
<td>6.4 ± 2.0</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>61-80</td>
<td>6.5 ± 1.2</td>
<td>25</td>
<td>7.2 ± 1.2</td>
<td>25</td>
</tr>
<tr>
<td>Cervical</td>
<td>21-40</td>
<td>8.3 ± 1.51</td>
<td>20</td>
<td>8.8 ± 1.2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>41-60</td>
<td>7.1 ± 0.8</td>
<td>28</td>
<td>7.2 ± 1.4</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>61-80</td>
<td>6.0 ± 1.6</td>
<td>15</td>
<td>7.9 ± 1.2</td>
<td>22</td>
</tr>
<tr>
<td>Ovarian</td>
<td>21-40</td>
<td>12.5 ± 1.3</td>
<td>12</td>
<td>10.8 ± 1.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>41-60</td>
<td>10.4 ± 1.6</td>
<td>16</td>
<td>10.5 ± 1.7</td>
<td>15</td>
</tr>
<tr>
<td>Breast</td>
<td>21-40</td>
<td>13.5 ± 2.9+</td>
<td>14</td>
<td>12.7 ± 1.54</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>41-60</td>
<td>12.4 ± 1.8</td>
<td>28</td>
<td>11.5 ± 2.00</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>61-80</td>
<td>9.5 ± 1.6</td>
<td>18</td>
<td>10.3 ± 1.8</td>
<td>15</td>
</tr>
</tbody>
</table>

n - in parentheses indicate number of subjects  \( P < 0.05 \)
<table>
<thead>
<tr>
<th>Type</th>
<th>Rural Male</th>
<th>Rural Female</th>
<th>Urban Male</th>
<th>Urban Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8.4 ± 1.2</td>
<td>7.8 ± 1.6*</td>
<td>7.7 ± 1.3</td>
<td>7.5 ± 1.5*</td>
</tr>
<tr>
<td>Colon</td>
<td>8.2 ± 1.0</td>
<td>7.5 ± 1.0</td>
<td>7.2 ± 1.2</td>
<td>7.1 ± 1.2*</td>
</tr>
<tr>
<td>Rectal</td>
<td>10.2 ± 1.5*</td>
<td>9.8 ± 1.2</td>
<td>11.5 ± 1.6*</td>
<td>10.6 ± 2.0*</td>
</tr>
<tr>
<td>Stomach</td>
<td>9.6 ± 1.1</td>
<td>9.2 ± 1.3</td>
<td>9.2 ± 1.8*</td>
<td>8.2 ± 1.2*</td>
</tr>
</tbody>
</table>

* P < 0.02

n - in parentheses indicate number of subjects

Note: The table is part of a study on serum arginase in colorectal cancer. The data are presented as mean ± standard deviation. The table compares serum arginase levels between different types of tumors and gender across rural and urban populations.
rurals than the urbans. The percent induction was 93%, 100% and 32% for rurals and 50%, 80% and 43% for urbans.

Similarly, the serum arginase levels were found to be slightly increased in rectal and stomach carcinomas (Table-2) and a little decrease was noted in colon cancer. The magnitude of induction was more in male subjects than females.

Follow up cases were not available in sufficient number and those available did not show in diseased subjects.

**DISCUSSION**

Serum arginase activity is increased in many patients with hepatitis and cirrhosis, but is within normal limits in obstructive jaundice and hepatoma (13). Serum levels are also normal in a variety of disease states including myocardial infarction, pneumonia, rheumatic fever and metastatic cancer, but elevated in 7 out of 9 cases of hepatic coma (14,15). Red cell arginase activity is invariably elevated in pernicious anaemia and
nutritional megaloblastic anemia returning to normal levels following successful treatment. Variations in leucocyte arginase activity apparently merely reflect the relative abundance of the neutrophils (6-7).

The urea cycle itself is not an independent unit. The oxidation of glucose beyond the process of glycolysis may be carried out by other enzyme systems to provide energy for the reactions. Interference with the activity of any other one enzyme, either directly or through coenzyme or other factor, may therefore have far-reaching effects, which, to judge by the signs and symptoms exhibited by the organism, are far removed from the immediate system in which the interference occurs. In many cases alternative metabolic pathways exist and while a deficiency or total lack of one enzyme may not in some instances give rise to any obvious evidence and thus abnormalities may not be compatible with life. Thus observed high enzyme levels in carcinomas may be due to disturbances in the metabolism as a whole as evidenced by similar high levels with LDH, phosphatase and 5'-nucleotidase.


   43: 581 (1948).

   87, 96 (1963).

5. Reynolds, J., Follette, J.H. and Valentine, W.N.,

6. Reynolds, J., Follette, J.H. and Valentine, W.N.


   95, 225 (1957).

9. Kumate, J., Benavides, L., Carillo, J., Santis, M., and

10. Shih, V.E., Jones, T.C., Levy, H.L. and Madigan, P.M.

    151, 711 (1957).

