INTRODUCTION

5'-Nucleotidase is an alkaline phospho-
monoesterase which differs from the nonspecific
serum alkaline phosphatase, in that its activity
is confined to the hydrolysis of nucleotide
pentose 5'-phosphate group only. It is a
plasma membrane marker enzyme in many mammalian
cells, where it is present as an ectoenzyme (1). 5'-nucleotidase (5'NT) was first discovered by
Reis (2).

Levels of the enzyme have been reported
to be altered in many disease states (3-6).
Dixon and Purdom (7) reported low levels of 5-NT
activity in osteogenic tumors, breast cancer and
spinal neoplasm. Reymenant and Tagnon (6) stated
that the diagnostic value of 5-NT determinations
could be advantageous in comparison to serum ALP,
because it was not influenced by bone diseases,
5-NT determinations may prove to be of
clinical value in view of the evidence that
it is at least as sensitive as serum
ALP in detecting the presence of biliary tract obstructions and is more selective because values are not increased in diseases of bone associated with increased osteoblastic activity.

A number of cellular functions such as cellular adhesiveness, contact inhibition of growth and movement and antigenicity are regulated by the cell plasma membrane. Transformed or malignant cells aberrant in these biologic characteristics, differ from their normal counterparts with respect to structure and composition of their plasma membrane. Plasma membrane constituents are shed into the surroundings media, *in vitro* and *in vivo*, as cells replicate.

Stojanovic *et al.* (8) reported elevated levels of serum 5'-nucleotidase in the patients suffering from endometrial cancers. Costanzo *et al.* (9) observed elevated levels of plasma glutamyltranspeptidase, leucine aminopeptidase and especially 5'-nucleotidase in secondary cancers of liver. It has been reported that some of colonic and rectal carcinomas had an elevated 5'-nucleotidase activity along with mammary carcinomas (10).
Raz et al. (11) reported decrease in the activity of 5'-nucleotidase in the patients suffering from leukemia. The decrease was 3 to 30 fold in malignant cells as compared to their parental cells. Similar results were observed by Goodlad et al. (12) in Walker 256 carcinoma. About 50% decrease in 5'-nucleotidase activity was reported in the liver. But Peter et al. (13) reported elevated levels of γ-Glutamyl transferase and 5'-nucleotidase in patients with liver metastases. About 58% of γ-glutamyltransferase and 40% of 5'-nucleotidase were above normals.

Wilson et al. (14) reported that 5'-nucleotidase levels were very low in various neoplastic organs of old and embryo C 57 and C3H mice. Bhatt et al. (15) studied distribution of 5'-nucleotidase in neoplastic nodules and tumors of the liver by inducing hexachlorocyclohexane (500 ppm) in Swiss mice for 2, 4 and 6 months. They reported higher 5'-nucleotidase activity in the tumor than the intact liver. Ihde et al. (16) reported elevated levels of 5'NT activity in 88.4% of 51 hepatoma patients. The value was about 10% more than normal limit. Kolaric et al. (17)
reported serum activities of liver LDH alkaline phosphatase, γ-glutamyltranspeptidase, and 5'-nucleotidase to be significantly elevated in malignant liver processes.

It is conceivable that solid tumors growing in a host would release membrane constituents into the systemic circulation. Due to proliferation of the tumor cell and higher metabolic rate than most normal cells, the rate of shedding into the circulation of a tumor bearing host would also be expected to be higher. Detection of released plasma membrane constituents in the circulation in amounts higher than normal might thus indicate the presence of tumor. In the search for a battery of biomarkers useful for diagnostic screening of carcinoma patients, 5'-nucleotidase was estimated in different carcinomas.

MATERIAL AND METHODS

Normal subjects were free of any known disease and were not taking any medication at the time of the study. All the normals were in the age group of 20 - 60 years. No significant effect
of age in the normal subjects was apparent from their serum 5'-nucleotidase levels. The total number of healthy subjects studied were 140.

The cancer subjects studied were the inpatients of Government Medical College and Hospital, Aurangabad. Histologically and radiologically proved carcinomatous patients were taken for the serological estimation of 5'-nucleotidase. The cancer group comprised of both rural and urban patients. The cancers studied were the cervical, ovarian, breast, colon, rectal and the stomach cancers. A description of the patients and their habitat, with their age group is given in the table - 1.

Collection of serum samples:

Blood was obtained by venipuncture method in the plain bulb. The blood samples were collected at 9.00 a.m. in the morning. After centrifugation for 10 minutes the sera was collected and utilized for the enzyme assay.
Assay of 5'-Nucleotidase:

The method of Campbell (1962) was utilized for the assay of 5'-nucleotidase (18). The reaction mixture contained 0.2 ml of serum, 1.5 ml of barbitone buffer (0.04 M), pH 7.5, 0.1 ml of manganous sulfate (0.02 M), 0.2 ml of nickel chloride MD and CAMP 0.2 ml (0.01 M). All incubation were carried at 37°C for 30 minutes. The reaction was terminated by the addition of 1.0 ml of TCA (10%). After centrifugation for 15 minutes the supernatant was utilized. To it 2.0 ml of acetate buffer (2.0 M, pH 4.0) was added followed by subsequent addition of 0.5 ml Metol (2%) and 0.5 ml ammonium molybdate. The color developed was measured at 660 nm on an Erma Colorimeter. Using Potassium dihydrogen orthophosphate (60 mM/L) as a standard.

RESULTS

5'-Nucleotidase activities in various normal and malignant subjects are presented in the table. Normal serum levels of 5'-nucleotidase were determined by assaying the enzyme in the sera of 141 healthy subjects. The levels ranged from 8.6-11.2 U/L.
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age</th>
<th>Rural</th>
<th>Urbans</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>140</td>
<td>21 - 40</td>
<td>8.6 ± 1.2</td>
<td>(15)</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>11.2 ± 0.6</td>
<td>(20)</td>
<td>10.8 ± 0.3</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>10.0 ± 0.4</td>
<td>(16)</td>
<td>10.0 ± 0.6</td>
<td>(25)</td>
</tr>
<tr>
<td>Cervical</td>
<td>135</td>
<td>21 - 40</td>
<td>18.88 ± 2.4+</td>
<td>(30)</td>
<td>11.93 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>28.60 ± 3.8+</td>
<td>(35)</td>
<td>9.10 ± 1.2</td>
<td>(30)</td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>27.21 ± 2.4</td>
<td>(15)</td>
<td>8.33 ± 2.1</td>
<td>(15)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>70</td>
<td>21 - 40</td>
<td>B 27.28 ± 4.2+</td>
<td>(10)</td>
<td>B 32.50 ± 2.6+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A 23.25 ± 1.6</td>
<td>A 25.42 ± 1.8</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90</td>
<td>25.83 ± 3.2+</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A 20.60 ± 2.5+</td>
<td>A 22.20 ± 2.0</td>
<td>(25)</td>
</tr>
<tr>
<td>Breast</td>
<td>90</td>
<td>21 - 40</td>
<td>8.60 ± 2.4</td>
<td>(12)</td>
<td>15.23 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>13.33 ± 1.6</td>
<td>(26)</td>
<td>14.59 ± 2.0</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>12.20 ± 1.2</td>
<td>A 12.20 ± 1.4</td>
<td>(10)</td>
<td>B 11.66 ± 1.5</td>
</tr>
</tbody>
</table>

A = After treatment;  
B = Before treatment;  
\( n = n \) in parentheses indicate number of subjects studied;  
\(+ p < 0.05\)
Most of the women subjects suffering from the genital cancers were in their stage III of the disease state. In patients with cervical carcinoma (n = 135), the serum levels of the enzyme with active disease were significantly higher in the rural subjects than in normals. The magnitude of induction was maximum in the age group of 61 - 80 years (172%). The same was not true for the urban subjects, though there was a slight increase in the enzyme levels when compared with the normal subjects. The percent elevation in the three age group was 32, 16 and 17 respectively.

In patients with ovarian carcinoma the enzyme level ranged from 26 - 32 U/L. The serum levels of 5'-nucleotidase in both the habitats were found to be significantly elevated than the normals. The percent induction was found to be more in the age group of 21 - 40 years than in 41 - 60 years. In the rural habitat the percent induction was 217 and 130 respectively. In the urban habitat the percent induction was 261 and 182 respectively. The number of patients for the follow up cases were not sufficient, although a fall in the enzyme levels was noted in the studied subjects.
In patients with breast carcinomas an increase was noted in the urban subjects. The percent induction noted in the rural subjects was 0, 19 and 58 respectively. In urban subjects the percent induction noted was 69, 35 and 16 respectively. Follow up cases indicated lowering up of the enzyme activity as compared to untreated patients.

In colon cancer 5'NT was elevated more in the female than the male subjects in both the habitats. The percent induction in the rural was 223 for the males and 298 for the females. In the urban the magnitude of induction was 310 and 417 respectively. Similar percent induction was noted in the patients suffering from stomach carcinomas. Though the induction was little lesser than the colon cancers. The percent induction for rural was 110 and 190 and in urban it was 219 and 230 respectively. In rectal cancers, the male of the urban habitat indicated highest rise in 5'NT to the extent of 100 whereas in rural habitat and female of urban the increase was within the range of 26 - 31. Follow up cases indicated significant alterations in the enzyme activity.
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Rural</th>
<th></th>
<th>Urban</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Normal</td>
<td>140</td>
<td>10.30 ± 2.2</td>
<td>9.20 ± 1.2</td>
<td>9.90 ± 1.5</td>
<td>8.90 ± 2.9+</td>
</tr>
<tr>
<td>Colon</td>
<td>55</td>
<td>B 33.33 ± 2.6+</td>
<td>(10) B36.66 ± 2.8+</td>
<td>(15) B40.66 ± 2.9+</td>
<td>(10) B46.00 ± 5.8+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 25.60 ± 1.8</td>
<td>A 24.80 ± 3.1+</td>
<td>A 33.90 ± 1.7</td>
<td>A 39.60 ± 2.6+</td>
</tr>
<tr>
<td>Stomach</td>
<td>47</td>
<td>B 21.66 ± 3.2+</td>
<td>(12) B26.66 ± 2.6+</td>
<td>(8) B31.66 ± 3.6+</td>
<td>(7) B38.33 ± 5.0+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 17.80 ± 2.0</td>
<td>A 20.60 ± 1.8</td>
<td>A 25.10 ± 2.5</td>
<td>A 32.50 ± 2.2</td>
</tr>
<tr>
<td>Rectal</td>
<td>50</td>
<td>B 13.33 ± 1.6</td>
<td>(11) B11.66 ± 1.2</td>
<td>(15) B20.00 ± 4.5+</td>
<td>(10) B11.66 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 12.40 ± 1.2</td>
<td>A 11.00 ± 1.2</td>
<td>A 14.80 ± 3.6+</td>
<td>A 10.40 ± 1.2</td>
</tr>
</tbody>
</table>

B = Before treatment
A = After treatment
n = in parantheses indicate number of subjects studied

+ P < 0.05
DISCUSSION

Plasma membrane marker enzymes are suitable candidates for such studies because a solid tumor growing in a tumor host sheds plasma membrane constituents into the systemic circulation of the host (19-20). Previous studies suggested that glycoprotein: galactosyltransferase (21-23) and cytidine 5'-monophospho-N-acetyl neuraminic acid hydrolase (24) are such enzymes.

In an animal model system for breast cancer, it was demonstrated that the activities of plasma membrane marker enzymes, including 5'-nucleotidase, were significantly reduced in the homogenates and purified plasma membrane preparations from the metastasizing tumors, compared with the nonmetastasizing controls. It was suggested that the reduction of the activities of plasma membrane marker enzymes were due to the shedding of these materials into the serum of the hosts. Diminished or undetectable levels of 5'-nucleotidase was also reported in the patients with chronic lymphocytic leukemia (25), which may be due to the shedding phenomenon.
Schwartz and Bodansky (26) determined 5'-nucleotidase in control groups and in patients with various types of neoplastic disease and found that the activity of the enzyme was elevated in the serum of cancer patients with hepatobiliary disease. However, there was no clear correlation between the level of this enzyme activity and other biochemical or clinical parameters of liver involvement. By sequential determinations of serum 5'-nucleotidase in two patients, they found that this enzyme can be useful in following the progression of carcinoma metastatic to the liver.

Boone et al. (77) found that serum 5'-nucleotidase is almost as useful as γ-glutamyltranspeptidase in indicating hepatobiliary disease in patients with granuloma of the liver, acute pancreatitis, congestive heart failure, rheumatoid arthritis, and carcinoma metastatic to the liver. Kim et al. (28) assessed the diagnostic value of measuring serum alkaline phosphatase, 5'-nucleotidase, glutamyltransferase, and glutamate dehydrogenase activities as an aid to the detection of liver metastasis. They found that
all these enzymes had diagnostic value, but 5'-nucleotidase appeared to have the greatest.

Koudsteal (10) reported elevated levels of serum 5'-nucleotidase in the patients suffering from colonic and rectal carcinomas, which correlate with our results on colon and rectal cancers.

The results of our study shows that malignancy in a number of cases is accompanied by increased synthesis of serum 5'-nucleotidase specially in the ovarian, colon and stomach carcinomas. The increased activity may be as a result of tumor progression thus releasing the enzyme in the systemic circulation. Different activities of this enzyme in different cancers give a reason to think that all these changes are secondary and depends on tumor progression. The increased 5'-NT activity in the above cancers can be utilized for the early diagnosis of cancer. Further studies of this enzyme are being done to determine whether 5'NT can detect ovarian and cervical carcinoma under circumstances when other markers fail.
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