CHAPTER SIX
Lipid Peroxides In Cancer.
INTRODUCTION

It is axiomatic that one cannot obtain the right answer until one has asked the right question. Most of our current knowledge of cancer interaction in particular, seems to consists of a vast accumulation of answers (clinical, experimental and epidemiological data) with little or no clear understanding of what the truly relevant question might be.

The occurrence, growth and development of cancer is dependent upon an altered lipid status, is one such hypothesis. The lipids are thought to modify cellular and membrane responses to other regulatory stimuli rather than to act as regulatory molecules per se. The corollary of this for the tumour is that the possession of even a slight defect in lipid status would profoundly affect cellular responsiveness.

Lipid peroxides can be formed by several mechanisms. Severe damage to the structural/ components of the cells is followed by auto oxidation of unsaturated fatty acids. On the other
hand, enzymes such as lipoxygenase, and cyclooxygenase catalyse the peroxidation of arachidonic acid leading to thromboxanes and prostaglandins.

The lipid peroxides formed by either mechanism are released from stimulated platelets. A variety of stimuli activate phospholipase A₂ resulting in the release of arachidonic acid from membrane phospholipids. Two distinct metabolic pathways transform this unsaturated fatty acids into different oxygenated products. The lipoxygenase pathway leads to the formation of several unstable products. The cyclooxygenase pathway leads to the formation of cyclic endoperoxides, thromboxanes A₂ and B₂ (1) and prostaglandins PG₂ and PGF₂ (2). Concomitantly, other end products of arachidonic acid metabolism are released which include malonaldehyde(3). Smith et al. (4) have shown that platelets produce malondialdehyde when stimulated by aggregating agents and they concluded that its estimation in plasma could be used as an indicator of platelet prostaglandin synthesis.
Prostaglandins, prostacyclins and leucotrienes, phosphatidylinositols, and gangliosides and a host of other lipids species yet untested, all display a significant association with rapid cell proliferation. Loss of control of synthesis of lipid is the only acceptable definition of the cancer state (5). For control of cancer, the lipidologists are arguing primarily on cholesterol and unsaturated fatty acids.

The process of lipid peroxidation takes through the interactions of free radicals and lipids (6). The process of lipid peroxidation is initiated by the reaction of oxygen with PUFAs to form free radical intermediate (7). Increase in these lipid peroxide results in the degeneration of organs or tissues (8-12). Lipid peroxides formed at the primary site are transferred through the circulation to other organs or tissues and provoke damage by propagating lipid products (13-14). Glavind et al. (15) reported that lipid peroxides occured in the plaques of human atheroma and that the degree of atheroma correlated with the extent of lipid peroxidation in the plaques. Fukuzumi (16)
demonstrated the occurrence of lipid peroxides in human atheromatous cell wall by infrared absorption technique and Aoyama and Iwakami (17, 18) by using rabbits from atherosclerosis by administration of cholesterol or lanolin. This was followed by the isolation of hydroperoxides of cholesterol linoleate from the lipids of advanced atherosclerotic plaques of human aortas by Hartland (19).

Sato et al. (20) observed that among diabetics, the patients with various kinds of angiopathy showed higher levels of plasma lipid peroxides in the subjects studied. Miki (21) reported that the blood lipid peroxides levels were increased in the patients suffering from atherosclerosis than the normal subjects.

Suematsu et al. (22) reported elevated levels of serum lipid peroxides in the cases of acute hepatitis, fulminant hepatitis, chronic active hepatitis and fatty liver when compared with normal subjects. Similarly the lipid peroxides levels were reported to be elevated in heavy drinkers than that in non-drinkers. Thus, high
levels of lipid peroxides in liver tissues may be related to the etiology of liver diseases. Jean Doussset et al. (23) determined malonaldehyde levels in plasma from patients with myocardial infarction which were found to be significantly high.

Shamberger et al. (24) demonstrated that malonaldehyde is one of the factor for tumorigenesis. He reported that malonaldehyde is a tumor initiator in promoting skin tumors in mouse. Shimmoyama et al. (25) reported that plasma lipid peroxides levels were increased in cancer patients, especially in cachetic state. Baur (26) reported high levels of malonaldehyde in carcinoma of colon when compared to the healthy tissue. Kozlov et al. (27) reported that a tumor in vivo in mice includes a loss of 

\[ \text{tocopherol} \]
by the liver which ceases to serve as a depot of vitamin \( \text{E} \), a potent antioxidant. As a result the liver of mice with Ehrlich carcinoma accumulates lipid peroxides.

Grueva (28) reported in tumor tissue of hamsters with pigmented melanoma, that the lipid peroxidation was lower in tumor tissue than in
healthy animals, and also in the liver microsomes of tumor carriers. Bartoli (29) also reported a decrease in the level of lipid peroxides in the neoplasm when compared with the normal. The decreased peroxidation products formation might represent one of the factors involved in uncontrolled neoplastic growth.

Carcinogenesis is a multi step process. Many cancerous cells may occupy living organisms without giving any clinical sign of cancer. Between carcinogenesis and the production of clinical cancer all the defenses of the organisms especially the immunological responses of the cell, may act. These cells may produce several types of free radicals when they contact their targets.

Several enzymes have been found to be altered during cancerous growth. These alterations could be related to the membrane lipids which are supposed to be the initiators of peroxide. Here we make an attempt to study the levels of serum lipid peroxides in various cancers in order to support the existing enzymic elevations in cancers.
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 subjects comprised of 51 rural and 90 urban. The controls were mostly the employees of Government Medical College and Hospital, Aurangabad. 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. These were further divided into 3 age groups, 21 - 40, 41 - 60 and 61 - 80 years (for cervical, ovarian and breast carcinomas). For colon, rectal and stomach cancers the groups were divided according to their sexes i.e. males and females, for both habitats.
Sample collection:

Blood samples were collected early in the morning in plain bulb, from the cubital vein. Serum was separated by centrifugation at 3000 rpm for 10 minutes, and the sample was analyzed for serum lipid peroxides by the method of Yagi (30).

Assay:

To 0.2 ml of serum, 4.0 ml of N/12 H₂SO₄ was added and mixed gently.

Then 0.5 ml of 10% phosphotungstic acid was added and mixed. After allowing it to stand at room temperature for 5 minutes, the mixture was centrifuged at 3000 rpm for 10 minutes.

The supernatant was discarded and sediment was mixed with 2.0 ml of N/12 H₂SO₄ and 0.5 ml of phosphotungstic acid. The mixture was centrifuged at 3000 rpm for 10 minutes.

The sediment was suspended in 4.0 ml of distilled water and 1.0 ml of 0.67% Thiobarbituric acid. The reaction mixture was heated for 60 minutes at 95° in water bath. After cooling, 5.0 ml of n-Butanol
was added and the mixture was shaken vigorously. After centrifugation at 3000 rpm for 15 minutes, the n-butanol layer was measured at 530 nm using tetramethoxy-propane (0-1.0 nmole) as standard.

The lipid peroxides levels were expressed in terms of malonaldehyde/ml.

RESULTS

Significantly low levels of serum lipid peroxide levels were observed in cancer patients when compared with the normal subjects, of the same age groups. Almost all values obtained were less than 3.5 nmol in terms of malonaldehyde (MDA) per ml. of serum. The urban normals indicated a greater MDA value than the rural normals.

Table - 1 indicates the lipid peroxide levels in the serum of patients suffering from cervical, ovarian, and breast carcinomas. The levels of lipid peroxides were lower than the control subjects. The magnitude of decrease was found to be more in the urbans than the rurals. The decrease was found to be more profound in the age group of 21 - 40 years.
### Table - 1

**Lipid peroxides in female genital cancers**

<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>2.5 ± 1.2*</td>
<td>(15)</td>
<td>3.5 ± 1.0*</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>1.6 ± 1.0</td>
<td>(20)</td>
<td>3.3 ± 0.7</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>2.05 ± 1.3*</td>
<td>(16)</td>
<td>3.4 ± 0.5</td>
<td>(25)</td>
</tr>
<tr>
<td>Cervical</td>
<td>21 - 40</td>
<td>B 0.95 ± 0.8</td>
<td>(25)</td>
<td>B 1.10 ± 0.4</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 1.56 ± 1.0</td>
<td></td>
<td>A 1.40 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>B 1.83 ± 1.0*</td>
<td>(40)</td>
<td>B 1.83 ± 0.5</td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 2.0 ± 1.0</td>
<td></td>
<td>A 2.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>B 1.2 ± 0.6</td>
<td>(30)</td>
<td>B 1.39 ± 0.4</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 1.8 ± 0.8</td>
<td></td>
<td>A 1.95 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>21 - 40</td>
<td>B 1.00 ± 0.5</td>
<td>(15)</td>
<td>B 1.39 ± 0.4</td>
<td>(30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 1.20 ± 0.6</td>
<td></td>
<td>A 1.68 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>B 2.05 ± 0.6</td>
<td>(30)</td>
<td>B 3.85 ± 1.0*</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 2.05 ± 0.2</td>
<td></td>
<td>A 3.95 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>21 - 40</td>
<td>B 1.39 ± 0.6</td>
<td>(25)</td>
<td>B 1.35 ± 0.4</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 1.56 ± 0.8</td>
<td></td>
<td>A 1.56 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>B 1.24 ± 0.2</td>
<td>(30)</td>
<td>B 2.7 ± 0.9</td>
<td>(35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 1.42 ± 0.2</td>
<td></td>
<td>A 2.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>B 2.9 ± 0.6</td>
<td>(20)</td>
<td>B 1.47 ± 0.6</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 2.8 ± 0.6</td>
<td></td>
<td>A 1.58 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

B = Before treatment;  
A = After treatment;  
n = Indicates number of subjects.  
* P < 0.01
for both the habitats of cervical cancers. The magnitude of decrease was 61%, and 66% in the rural age groups and 68%, 45% and 59% in urban. The percent decrease indicates some effect of the disease on the age.

Serum lipid peroxide levels were also found to be decreased in ovarian tumor subjects than the healthy subjects. The decrease was more significant in the age group of 21 - 40 years. The percentage decrease was more than 60% for both the habitats. In the age group of 41 - 60 years a slight increase was noted for both the habitats.

In breast cancer also a decline was noted in serum lipid peroxide levels when compared with the healthy subjects. The percent decrease noted for rural age group was 48 and 25 for 21 - 40 years and 41 - 60 years respectively. In the urban age group the percent decline was found to be 62, 18 and 58 respectively.

Similarly, the levels of lipid peroxides were found to be decreased in stomach, colon and
Table - 2

Serum Lipid peroxides in colorectal cancer/GIT

<table>
<thead>
<tr>
<th>Type</th>
<th>Male n</th>
<th>Rural Female n</th>
<th>Urban Male n</th>
<th>Urban Female n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.1 ± 0.2 (25)</td>
<td>2.05 ± 0.9* (30)</td>
<td>3.6 ± 1.0* (35)</td>
<td>3.4 ± 1.2* (35)</td>
</tr>
<tr>
<td>Colon</td>
<td>1.9 ± 0.2 (12)</td>
<td>1.60 ± 0.4 (18)</td>
<td>1.8 ± 0.6 (15)</td>
<td>1.5 ± 0.8* (15)</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.6691 ± 0.6 (16)</td>
<td>0.80 ± 0.4 (14)</td>
<td>0.588 ± 0.4 (15)</td>
<td>0.43 ± 0.4 (15)</td>
</tr>
<tr>
<td>Rectal</td>
<td>2.10 ± 0.2 (12)</td>
<td>1.50 ± 0.5 (10)</td>
<td>1.8 ± 0.8* (16)</td>
<td>1.385 ± 0.3 (14)</td>
</tr>
</tbody>
</table>

B - n - in parantheses indicate number of subjects.  
* P<0.01
rectal carcinomas (Table-2). The magnitude of decrease was more in the female than male. Furthermore, the magnitude of decrease was highest with stomach cancers and lowest with rectal cancers irrespective of the sex.

Short term follow up cases did not indicate significant change in the lipid peroxide levels in either of the cancer subjects.

**DISCUSSION**

Egyud and Gyorgy (33) and Schauenstein et al. (34), observed that tumor cells generally have a very low rate of lipid peroxidation in comparison to normal which have considerable peroxidizing activity. Grueva observed lower levels of lipid peroxides in the tumor tissue of hamsters with pigmented melanoma when compared to healthy animals. He also observed high glutathione peroxidase activity in the tumor tissue.

Our results reported in the present studies are supported by similar observations reported by Slater (35) in tumor cells. The reason underlying
such decreases in cancer cells may be due to changes in the membrane contents of polyunsaturated fatty acids (PUFA), or due to decreases in free radical initiation reactions or may be due to increase in the tissue antioxidant content which would unable the tumor cells to peroxidize easily. This could indicate that the safety mechanism has been lost, perhaps by an increased ability of tumor cells material to scavenge the peroxyl radicals, or changes in the rates of metabolism of the aldehyde products.

Enzyme glutathione (GSH) peroxidase may be responsible for such decline in serum lipid peroxide due to its scavenging property by removing hydrogen peroxide and hereby slowing down hydrogen peroxide dependent free radical attack of the lipids. This conclusion was clearly supported by the protective activity of GSH peroxidase, but also of catalase and superoxide dismutase, thus scavenging the free radicals. This suggests that the growth of tumor in the body is preventing the release of lipid peroxides in the bloodstream. Thus low levels of serum lipid peroxides could serve as an indicator/
suspector for tumor formation in the body
when other enzymes levels are significantly
elevated.

The human being has one of the largest life
spans, which can indicate that during evolution
an effective protective mechanism against
oxygen radicals has been developed. Ames et al.
(36) reported that uric acid is inhibitory to
lipid peroxidation and they pointed out uric
acid as being one of the systems which protects
against oxygen radicals. Beyond any doubt, man
has among the mammals one of the highest
concentration of uric acid. From physiological
point these changes in the membrane are very
important with respect to the ageing of erythrocytes.
It had been observed in few cases that radio-
therapy enhances release of hydrolytic enzymes
from lysosomes which correlated with the
malonaldehyde content. Thus the agents which
inhibited lipid peroxidation enhanced lysosomal
enzyme release in parallel suggested that both
events are closely related.
REFERENCES


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SUMMARY AND CONCLUSIONS
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Serum enzyme determinations have become a major laboratory aid in the diagnosis of many diseases. The usefulness of knowledge of the serum enzymes results from their origin in cells of the tissue that may be affected by the disease process, i.e. enzymes are tissue markers and serve as indicators of cellular destruction.

During the period of December 1980 to June 1984, serum levels of alkaline phosphatase, acid phosphatase, 5'-nucleotidase (5'-NT), lactate dehydrogenase arginase and lipid peroxides in patients with various carcinomas were closely followed in an attempt to determine if these measurements had clinical value in predicting the course of patients with the disease.

Serum alkaline phosphatase was elevated in almost all the cancers studied with varying frequency. The elevation thus may be due to the primary tumor formation or due to distant metastases. Enzyme concentration of a primary tumor is generally high.
The elevation of various enzymes studied in malignant tumors give a reason to think that all these changes are secondary and depend on tumor progression. Thus it is evident that the assay of one enzyme is not enough for the diagnosis of cancer, assay of several enzymes may, however, be helpful in some cases. Interpretation of the levels of different enzymes involves knowledge of the various sites of production, the cause of stimulation or enzyme induction and the mechanism of release in various disease states.

The findings suggested that the changes of lactate dehydrogenase in serum of patients with malignant tumors are an expression of fundamental metabolic changes in the total organism. The elevated levels of different enzymes in the process of malignancy in a number of cases may be accompanied by increased synthesis of the studied enzymes. Follow up cases indicated low survival rate even though enzyme levels were nearing their normal. This indicates the alteration of the entire metabolic set up of the patient. Thus it is not fair to dismiss
all biochemical tests and all enzyme tests as having no place in cancer diagnosis. The biochemist, radiologist and cancer therapist should create chemical tools for the specified delivery to tumor cells of radionuclides that will detect them and drugs that will kill them.