CHAPTER - 1

General Introduction

The dreaded disease Cancer speaks:

"I am the world's second largest killer;

Characterized by uncontrolled proliferation of cells;

Implicating environmental and genetic factors;

perhaps due to oncogene and antioncogene imbalance."

The body is made up of hundreds of millions of living cells. Normal body cell grow, divide and die in an orderly fashion. During the early years of a person’s life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out dying cells or to repair injuries.

Cancer begins when cells in a part of the body start to grow out of control. There are many kinds of cancer, but they all start because of out-of-control growth of abnormal cells. Cancer cell growth is different from normal cell growth. Instead of dying, cancer cells continue to grow and form new, abnormal cells. Cancer cells can also invade (grow into) other tissues, something that normal cells cannot do. Growing out of control and invading other tissues are what makes a cell a cancer cell.

Cells become cancer cells because of damage to DNA, which is present in every cell and directs all its actions. In a normal cell, when DNA gets damaged the cell either repairs the damage or the cell dies. In cancer cells, the damaged DNA is not repaired, but the cell doesn’t die like it should. Instead, this cell goes on making new cells that the body does not need. These new cells will all have the same damaged DNA as the first cell does.
People can inherit damaged DNA, but most DNA damage is caused by mistake that happens while the normal cell is reproducing or by something in our environment. Sometimes the cause of the DNA damage is something obvious, like cigarette smoking. But often no clear cause is found. In most cases the cancer cells form a tumour. Some cancers, like leukemia, rarely form tumours. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow.

Cancer cells often travel to other parts of the body, were they begin to grow and form new tumours that replace normal tissue. This process is called metastasis. It happens when the cancer cells get into the bloodstream or lymph vessels of our body. No matter where a cancer may spread, it is always named after the place where it started. For example, breast cancer that has spreaded to the liver is still called breast cancer, not liver cancer.¹

The International Union against Cancer, UICC [Union for International Cancer Control] has defined cancer as disturbance of growth characterized by excessive proliferation of cells without apparent relation to physiological demands of organs involved. Oncology deals with the etiology, diagnosis, treatment, prevention and research aspects of Cancer. ²

Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignancy, malignant tumours and neoplasms. One defining feature of Cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries and which can then invade adjoining parts of the body and spread to other organs - i.e. secondaries. This process is referred to as Metastasis. ³
Carcinoma (Cancer) is derived from the Greek word meaning a "CRAB". This term was probably due to the appearance of large prominent veins surrounding the growth which resemble the claws of a crab. Today's modern health science is well equipped with advanced Light and Electron Microscopy and other instrumentation. Such facilities were not in existence during period of Sushruta. This ancient Indian surgeon expressed his views regarding this disease as a swelling situated either superficially or in deeper structure in relation to different systems and organs. His views depended entirely on “Tridosha and Dhatu” theory which is based entirely on clinical manifestations, course, prognosis and treatment available in those periods. \(^{(3)}\)

The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries. Based on the GLOBOCAN 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008, of these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths. Lung cancer is the leading cancer site in males, comprising 17% of the total new cancer cases and 23% of the total cancer deaths. Breast cancer is now also the leading cause of cancer death among females in economically developing countries, a shift from the previous decade during which the most common cause of cancer death was cervical cancer. Further, the mortality burden for lung cancer among females in
developing countries is as high as the burden for cervical cancer, with each accounting for 11% of the total female cancer deaths. Although overall cancer incidence rates in the developing world are half those seen in the developed world in both sexes, the overall cancer mortality rate are generally similar. Cancer survival tends to be poorer in developing countries, most likely because of a combination of a late stage at diagnosis and limited access to timely and standard treatment. A substantial proportion of the worldwide burden of cancer could be prevented through the application of existing cancer control knowledge and by implementing programs for tobacco control, vaccination (for liver and cervical cancer), and early detection and treatment, as well as public health campaigns promoting physical activity and a healthier dietary intake. Clinicians, public health professionals, and policy makers can play an active role in accelerating the application of such interventions globally. (4)

These statistics are based on GLOBOCAN 2008, the standard set of worldwide estimates of cancer incidence and mortality produced by the International. (5)

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries worldwide. According to World Health Organization, Cancer accounted for 7.6 million deaths [around 13% of all deaths] in 2008. (4)

The burden of cancer is increasing in economically developing countries as a result of population ageing and growth as well as, increasingly, an adoption of Cancer associated lifestyle choices including smoking, physical inactivity, and "westernized" diets. (3,6)

The main types of cancer and resulted deaths are [as adapted from Reference no. 2]
Lung - 1.4 million deaths
Stomach - 7,40,000 deaths
Liver - 7,00,000 deaths
Colorectal - 6,10,000 deaths
Breast - 4,60,000 deaths

Global Problem in Cancers:

Total Cancer/ Cases: 10.1 million
Men: 3.85 million
Women: 3.77 million
Developed countries: 4.7 (48%) million
Developing countries: 4.4 (52%) million
Projected Cancer Cases by 2020: 15.3 million

Cancer Incidence in India:

- Males -110/1,00,000
- Females -120/1,00,000
- Estimated cases of cancer - 8,00,000/year
- One in every 15 men, one in every 12 women develop Cancer during their lifetime.

Common Facts of Cancer:

- Males-Tobacco related Cancer.
- Females - Cervix and breast-cancer constitute 40%
- Stomach cancer is predominant in Bangalore and Chennai.
- More than 80% of cancers are lifestyle related.
- 70% of cancer occur during 35-64 years age (3).
Figure No. 1.2

Global Cancer Statistics\(^{(4,5)}\)

<table>
<thead>
<tr>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Worldwide</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Lung</td>
<td>Breast</td>
</tr>
<tr>
<td>495,000</td>
<td>1,488,560</td>
</tr>
<tr>
<td>Stomach</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>640,000</td>
<td>130,000</td>
</tr>
<tr>
<td>Liver</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>320,000</td>
<td>33,000</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Stomach</td>
</tr>
<tr>
<td>520,000</td>
<td>290,000</td>
</tr>
<tr>
<td>Bladder</td>
<td>Corp. &amp; gen.</td>
</tr>
<tr>
<td>240,000</td>
<td>263,000</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>Brain</td>
</tr>
<tr>
<td>240,000</td>
<td>225,000</td>
</tr>
<tr>
<td>NHL</td>
<td>Kidney</td>
</tr>
<tr>
<td>140,000</td>
<td>150,000</td>
</tr>
<tr>
<td>All sites but skin</td>
<td>NHL</td>
</tr>
<tr>
<td>1,530,000</td>
<td>1,510,000</td>
</tr>
<tr>
<td><strong>Developed Countries</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Breast</td>
<td>Lung &amp; bronchus</td>
</tr>
<tr>
<td>402,000</td>
<td>357,500</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Stomach</td>
</tr>
<tr>
<td>132,000</td>
<td>110,000</td>
</tr>
<tr>
<td>Liver</td>
<td>Corp. &amp; gen.</td>
</tr>
<tr>
<td>162,000</td>
<td>170,000</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>All sites but skin</td>
</tr>
<tr>
<td>35,000</td>
<td>6,000</td>
</tr>
<tr>
<td>NHL</td>
<td>Bladder</td>
</tr>
<tr>
<td>85,000</td>
<td>70,000</td>
</tr>
<tr>
<td>Uterus</td>
<td>Ewing</td>
</tr>
<tr>
<td>40,000</td>
<td>40,000</td>
</tr>
<tr>
<td>All sites but skin</td>
<td>NHL</td>
</tr>
<tr>
<td>1,540,000</td>
<td>1,520,000</td>
</tr>
<tr>
<td><strong>Developing Countries</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>Breast</td>
</tr>
<tr>
<td>370,000</td>
<td>165,000</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>Colon &amp; rectum</td>
</tr>
<tr>
<td>230,000</td>
<td>130,000</td>
</tr>
<tr>
<td>Stomach</td>
<td>Liver</td>
</tr>
<tr>
<td>770,000</td>
<td>305,000</td>
</tr>
<tr>
<td>Corp. &amp; gen.</td>
<td>Corp. &amp; gen.</td>
</tr>
<tr>
<td>160,000</td>
<td>160,000</td>
</tr>
<tr>
<td>Uterus</td>
<td>Uterus</td>
</tr>
<tr>
<td>160,000</td>
<td>160,000</td>
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<tr>
<td>NHL</td>
<td>NHL</td>
</tr>
<tr>
<td>520,000</td>
<td>520,000</td>
</tr>
<tr>
<td>All sites but skin</td>
<td>All sites but skin</td>
</tr>
<tr>
<td>2,440,000</td>
<td>2,440,000</td>
</tr>
</tbody>
</table>

Multi-Hit Theory of Cancer:

An alteration in one single gene is not typically enough to cause cancer in humans. Instead, cancer is believed to occur when we accumulate multiple alternations. In genes that are part of pathways critical to cell growth and the regulation of normal cell behavior.

When a cell accumulates 4 to 6 such mutations, it may begin to replicate out of control, eventually resulting in cancer. These cells lose their shape and become unable to perform their normal functions. Because cancerous cells are very good at replicating and growing, they crowd out normal cells in the tissue, preventing them from performing their functions. Some cells can escape into the blood and be transported to distant locations where they begin to replicate uncontrollably. In this case, the tumour is said to be metastatic (8,11).

Figure 1.3
Multi Hit Theory of Cancer
**Etiology of Cancer:**

All Cancers are multifactorial in origin including genetic, hormonal, metabolic, physical, chemical and environmental factors. All Cancers originate usually from a single aberrant cell which multiplies and produces a tumour mass. One mutation occurs out of $10^6$ divisions. By the time person reaches adulthood, $10^{26}$ cell divisions have occurred. It means $10^{20}$ mutations have already been produced in adult individual. Even if one out of every $10^{10}$ mutations is cancerous, this leads to $10^{16}$ transformed cells. Thanks to our immune system as these aberrant cells are usually destroyed by them. As age advances, probability of cancer incidence increases as immunity is decreased. So it is disease of old age i.e. after 60 years. But today this fact is changing due to our lifestyle. Cancer is the second most common cause of death after Cardiovascular disease (1,7).

<table>
<thead>
<tr>
<th>Table 1.1</th>
<th>Relationship of life expectancy with Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Developed Countries</td>
</tr>
<tr>
<td>Average life span</td>
<td>67 years</td>
</tr>
<tr>
<td>Death due to cancer</td>
<td>25%</td>
</tr>
</tbody>
</table>
### Table 1.2

**Incidence of Neoplasms in India and developed Countries**

<table>
<thead>
<tr>
<th></th>
<th>India</th>
<th>Developed Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong>:</td>
<td>Oral - 30%</td>
<td>Oral - 2%</td>
</tr>
<tr>
<td></td>
<td>Pharynx -10%</td>
<td>Lung – 35%</td>
</tr>
<tr>
<td></td>
<td>Oesophagus - 6 %</td>
<td>Colon and Rectum - 25 %</td>
</tr>
<tr>
<td><strong>Females</strong>:</td>
<td>Cervix - 30%</td>
<td>Cervix - 10%</td>
</tr>
<tr>
<td></td>
<td>Oral - 18%</td>
<td>Colon and Rectum - 25%</td>
</tr>
<tr>
<td></td>
<td>Breast - 16%</td>
<td>Breast - 35%</td>
</tr>
</tbody>
</table>

(Adapted from Reference No.1,7)

Cancer is a class of diseases characterized by out of control cell growth. There are over 100 different types of cancer and each is classified by the type of cell that is initially affected. Cancer harms the body when damaged cells divide undoubtedly to form lumps or masses of tissue called tumours [except in leukemia where cancer prohibits normal blood function by abnormal cell division in blood stream]. Tumours can grow and interfere with the digestive, nervous and circulatory system and they can release harmones that alter body functions. These in turn alter the enzymatic activities and bring changes in their concentrations. Such enzymatic alterations are studied in this research.
Cancer arises from one single cell. The transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumors. These changes are the result of the interaction between a person’s genetic factors and three categories of external agents including as follows.

**Etiology of Cancer (Carcinogenesis) can be studied under two heads(1)**

I Predisposing factors and

II Carcinogenic agents (Agents Causing Cancer)

1. Physical – Radiant energy
2. Chemical – Variety of chemical compounds can cause cancer, some act directly and others act as procarcinogens.
3. Biological – Oncogenic viruses

**Predisposing Factors are as follows:**

1. **Age**: Cancer can develop in any age but is most common in those over 55 years age. Certain cancers are practically common in children below 15 years age viz – retinoblastoma’s, neuroblastomas, Wilm's tumor, certain tumors of haemopoietic tissues as lymphomas and leukaemias, sarcomas of bones and skeletal muscles.

2. **Heredity**: Heredity plays an important role in carcinogenesis. Certain precancerous conditions are inherited eg. susceptibility to childhood retinoblastomas is inherited as an autosomal dominant trait and approximately 40% of retinoblastoma are familial. Retinoblastoma – It has been recently studied that Eye cancer in kids may be due to mom’s cervical cancer. Doctors study, whether if HPV is responsible for retinoblastoma. It is believed that retinoblastoma is caused by genetic mutation. More than half of the cases though don’t have family history of eye cancer, HPV is a suspect in these cases (Dr. Sangeetha Desai, *The Times of India*, March 15, 2012.)
3. **Environmental Factors** – Statistically it has been shown that 80% of human cancer are caused by environmental factors, principally chemical.

Viz –

- **Lifestyle** : Cigarette smoking, Tobacco chewing.
- **Dietary** : Groundnuts and other food-stuffs
- **Occupational** : Asbestos, benzene, naphthylamines.
- **Iatrogenic** : Certain therapeutic drugs may be carcinogenic

4. **Acquired Precancerous Disorders** :

Certain clinical conditions are associated with increased risk of developing cancer. Examples are

- **Leukoplakia** – of oral mucosa and genital mucosa develops into squamous cell carcinomas.
- **Cirrhosis of Liver** – A few cases can develop hepatocellular Carcinoma (Hepatoma)
- **Ulcerative Colitis** – Can produce adenocarcinoma of colon
- **Carcinoma in situ of cervix** – Can produce squamous cell carcinoma of cervix.

II.1. **Physical carcinogens or Radiant Energy (Radiations) : UV and ionizing radiations** :

Ultra violet rays (uv rays), X-rays and γ-rays are mutagenic and carcinogenic. These rays damage DNA in several ways, UV radiations cause formation of pyrimidine dimers. Apurinic or apyrimidinic sites may form by elimination of corresponding bases. Single and double strand breaks or cross- linking of strands may occur. Apart from direct effect of DNA, X-rays & γ-rays cause free radicals to form in tissues. Resultant hydroxyl (OH), superoxide and other radicals can interact with DNA and other macromolecules leading to molecular damage thus contributing carcinogenic effect of radiant energy.
**Ionizing radiation:**

The ability of ionizing radiations to cause cancer lies in their ability to produce mutations. Particulate radiations such as $\alpha$-particles and neutrons are more carcinogenic than electromagnetic radiations like X-rays and $\gamma$-rays. In favour of this carcinogenicity of ionizing radiations following evidences can be stated –

- Incidence of leukemia increased in Japan after atom bomb explosion.
- Development of thyroid cancer in later life in children exposed to therapeutic radiation in neck is seen.
- Lung cancer is more in miners who work in radioactive ore mines.

**II.2 Carcinogenic Agents (Agents causing cancer):**

80% of human cancers are caused by environmental factors mainly by chemicals (1). These factors may be introduced into the body by means of

- a. Diet : eg. Aflatoxins or
- b. Lifestyle : eg. Smoking

Chemical carcinogens act cumulatively. Tobacco, food additives, coloring agents and aflatoxins are common carcinogens in our environment. Aflatoxin B$_1$, produced by mould *Aspergillus flavus* is sometimes found as contaminant of peanut’s and other food, acts as a chemical carcinogen. Cigarette contains many carcinogens, the most important group being benzopyrenes. Other important deleterious substances in smoke are nicotine, carbon- monoxide, nitrogen-dioxide and carbon soot. Nicotine is a natural poison, increases heart rate and blood pressure. It may produce transient contraction of coronary artery which may trigger a thromboembolic attack in coronary vessels. Cigarette smoke contains 5% carbon monoxide which depletes oxygen in red blood cells.
and restrict oxygen availability to vital areas including heart. Nitrogen-dioxide content in cigarette smoke is 250 ppm, as compared to safe industrial level of 5 ppm. It corrodes cellular membrane of alveolar sacs, leading to emphysema. The carbon soot decreases ciliary activity of nasopharyngeal tract. Carbon particles powerfully suppress the macrophage function. Statistically it is estimated that one cigarette reduces 10 minutes from the life span of an individual. A large number of chemicals have been incriminated as carcinogenic. Some of these are direct reacting and majority occur as pro-carcinogens which are converted in the body to ultimate carcinogenic chemicals.

### II.2 Carcinogenic Agent (Agents Causing Cancer):

As described earlier carcinogens can be divided into three main broad groups (1).

a. Physical – radiant energy

b. Chemical – Variety of chemical compounds can cause cancer, some act directly and others act as procarcinogens.

c. Biological – Oncogenic viruses
### Table 1.3
Some important Chemical Carcinogens

<table>
<thead>
<tr>
<th>CLASS</th>
<th>COMPOUND</th>
<th>NOTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Polycyclic aromatic hydrocarbons</td>
<td>Benzopyrene, Dimethyl benzanthracene (DMBA)</td>
<td>Hydrocarbons are present in cigarette smoke and are thus relevant in pathogenesis of lung cancer.</td>
</tr>
<tr>
<td>2. Aromatic amines (azo – dyes)</td>
<td>2-Acetylamino fluorine, β-Napthylamine, N-methyl-4-aminoazo benzene</td>
<td>β-Napthylamine used in rubber industries results for bladder cancers in exposed workers.</td>
</tr>
<tr>
<td>3. Nitrosamines and amides</td>
<td>Dimethyl nitrosamine</td>
<td>Nitrosamines and amides can be synthesized in GItract from injected nitrites and contribute to induction of gastric cancer.</td>
</tr>
<tr>
<td>4. Various drugs</td>
<td>Alkylation agents</td>
<td>Are used in cancer treatment, such patients are at high risk for developing cancer.</td>
</tr>
<tr>
<td></td>
<td>o Cyclophosphomide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o busulphan</td>
<td></td>
</tr>
<tr>
<td>5. Naturally occurring compounds</td>
<td>o Dactinomycin</td>
<td>Aflatoxin B1 is related with liver cell carcinoma in Africa</td>
</tr>
<tr>
<td></td>
<td>o Aflatoxin B1</td>
<td></td>
</tr>
<tr>
<td>6. Inorganic Compounds</td>
<td>o Arsenic, Asbestos, Beryllium, Saccharin</td>
<td>Saccharin acts as “promoter”</td>
</tr>
</tbody>
</table>
Three important chemical carcinogens are:

1. Benzopyrene
2. 2 - Acetylaminofluorine
3. N- methyl - 4 aminoazobenzene

Biochemical Aspects

Mechanism of Chemical Carcinogenesis:

As discussed above, chemical carcinogens may be:

a. Direct acting
b. Procarcinogens

a. Direct acting:
   Nitrogen, mustard and \( \beta \)-propiolactone directly interacts with target molecules, thus are direct carcinogens.

b. Procarcinogens:
   Unlike direct carcinogens, some chemicals require prior metabolism to become carcinogenic, these are Procarcinogens.

Process whereby one or more enzyme catalyzed reactions convert procarcinogens to active carcinogens is called metabolic activation. Any intermediate compounds formed are Proximate Carcinogens and final compound that which with cellular components eg. DNA is Ultimate Carcinogen. This sequence can be represented schematically as follows.

Fig. 1.4

Metabolic Activation of Procarcinogen

<table>
<thead>
<tr>
<th>Procarcinogen</th>
<th>Proximate carcinogen A</th>
<th>Proximate carcinogen B</th>
<th>Ultimate carcinogen</th>
</tr>
</thead>
</table>

Procarcinogen is a non-reactive species. Ultimate carcinogen is highly reactive. They are usually electrophiles i.e. molecules deficient in electrons, which readily attack nucleophilic (electron rich) groups in DNA, RNA and proteins.
Metabolism of Chemical Carcinogens:

Metabolism of procarcinogens and other xenobiotics involve the cytochrome P_{450} species enzyme system located in the Endoplasmic reticulum of cells mainly Mono-oxygenase and Transferases\(^{(1,7)}\). The activities of enzymes metabolizing carcinogens are affected by number of factors such as species, genetic consideration, age and sex. Intake of Phenobarbitol PCBS, or certain hydrocarbons can also increase the activities of many enzymes by a process known as Enzyme-induction. Hydrocarbon inhalation from cigarette during pregnancy induces the activity of cytochrome P_{448} in the placenta altering the amounts of certain metabolites of hydrocarbons to which the foetus is exposed. Metabolites of certain drugs also can inhibit activities of xenobiotic-metabolizing enzymes.

First mechanism of action of chemical carcinogens being ultimate carcinogens, the second mechanism is Covalent Binding. When chemical carcinogens are administered to animals or placed in cultured cells, they or their derivatives bind covalently to cellular macromolecules, DNA,RNA and proteins. Carcinogen interacts with purine, pyrimidine or phosphodiester group of DNA. Most common sites of attack are guanine residues and \(N_2, N_3, N_7, O_6\) and \(O_8\) atoms of this base. They are highly prone to addition of carcinogen group.

Biochemical Basis : Damage to DNA:

Covalent interaction of direct or ultimate Carcinogens with DNA result in several types of damages which can be repaired. Inspite of existence or repair system, certain modifications of DNA by chemical carcinogens persists for relatively long periods. It is possible that these persistant unrepaired lesions are of special importance in generating mutations critical to carcinogens \(^{(1,7)}\).
**Mutagens:**

Most chemical carcinogens are Mutagens. At molecular level, transition, transversion and other types of mutations occur on exposure of certain bacteria to ultimate carcinogens. Some types of cancers are due to mutations in somatic cells that affects key regulatory processes in these cells.

**Antimutagens:**

There are substances which interfere with tumor promotion, few of them are as follow –

**Vitamin ‘A’ and Carotenoids:**

They reverse precancerous conditions, specially oral leukoplakia. Thus in this research study further we see that the quantity of vitamin ‘A’ is decreased in the serum with increased diseased condition. Studies shows that supplementation of vitamin A in such cases reversed the pre cancerous stage.\(^{(3,9)}\)

BHA (Butylated Hydroxy Anisole), a preservative in cooking oils, acts as an antioxidant. Vitamin ‘C’ and Vitamin ‘E’ also act as antioxidants, preventing the damage made by free radicals and superoxides. Workers in factories producing aniline dyes, are prone to develop bladder cancer due to ingestion of this carcinogen. Vitamin ‘C’ regularly given to such workers prevented the production of new cancer cases by reduction of the dye into safer products Turmeric is known to prevent mutations because of the yellow substance curcumin present in it\(^{(9)}\).

Asbestos, components of tobacco smoke, aflatoxin (a food contaminant) and arsenic (a drinking water contaminant) are some of the carcinogens.
II.3 Biological Carcinogenes - Viral Oncongenesis:

Another probable etiological factor of carcinogenesis is the integration of viral genes into the host DNA. Viruses that produce tumours in their natural hosts or in experimental animals or induce malignant transformation of cells in culture, are known as Oncogenic Viruses (1,7).

The association of viruses with malignancy dates from the observation by Ellermen and Bang [1908] that the mode of transmission in leukemia in fowls resembled that of an infectious disease. Rous [1911] showed that a solid malignant tumour, fowl sarcoma was caused by a virus, a discovery for which he was awarded the Noble Prize in 1966 at his age of 85 years. Viruses causing tumours in animals were first demonstrated by Shope, in 1932. Many viruses have been isolated from human cancers or demonstrated electro microscopically in affected cells and tissues but most of them are nearly "passenger" viruses present in the lesions and not the causative agents. It is now acknowledged that virus infections account for 10 to 20 percent of human malignancies. These include hepatocellular carcinoma caused by Hepatitis B or C viruses, uterine cervical cancer by certain types of papilloma viruses, anaplastic nasopharyngeal carcinomas by the EB (Epstein-Barr) virus and adult cutaneous T-cell lymphoma / Leukemia by HTLV - 1 (Human T-cell Leukemia Virus-1)

The oncogenic viruses induce malignant transformation which accompanies various changes while the conversion of a normal cell into a malignant cell. This transformation from normal to malignant cell is a multistep process and may be partial or complex.

Properties of cells transformed by viruses:

1. Altered cell morphology: Fibroblasts become shorter, parallel orientation is lost, chromosomal aberrations appear.
2. **Altered cell metabolism**: Increased growth rate, increased production of organic acids and acid mucopolysaccharides.

3. **Altered growth characteristics**: Loss of contact inhibition, formation of heaped-up growth [microtumours], capacity to divide indefinitely in serial culture, capacity to grow in suspension or in semisolid agar.

4. **Antigenic alterations**: Appearance of new virus specified antigens (T-antigen - TSTA), loss of surface antigens, cells become agglutinable by lectins.

5. Capacity to induce genomes in susceptible animals.

**Six Hallmarks of Cancer Cells:**

1. Self sufficient growth signals
2. Insensitivity to signals that inhibit growth
3. Ability to evade programmed cell death (apoptosis)
4. Ability to replicate indefinitely
5. Ability to generate sustained blood supply (angiogenesis)
6. Ability to invade tissue and metastasize (spread to other areas) \(^{(8,10)}\)

**Oncogenes:**

Oncogenes are genes capable of causing cancer (Greek-Oncos: tumour or mass) these were first recognized as unique–genes of tumour–causing viruses that are responsible for the process of transformation (Viral Oncogenes). Oncogene is a mutated form of a gene involved in normal cell growth. In their normal, unmutated state, oncogenes are called proto-oncogenes, and they play roles in the regulation of cell division. Some oncogenes work like putting your foot down on the accelerator of a car, pushing a cell to divide. When the oncogene is activated, it is similar to having the accelerator stuck to the floor.\(^{(3)}\)
Oncogenes of Rous Sarcoma Virus (RSV):

Oncogenes of Rous Sarcoma Virus (RSV) are well studied\(^{(1,7)}\)

1. The genome of this retrovirus contains four genes named gag, pol, env and src.
2. The gag gene codes for group-specific antigens of the virus, pol for the reverse transcriptase that characterizes retroviruses and env for certain glycoproteins of the viral envelope. A protein-tyrosine kinase is seen to be the product of src (i.e. the sarcoma causing gene) that is responsible for transformation.
3. Certain glycolytic enzymes become target proteins for the src protein-tyrosine kinase. This shows that transformed cells often show increased rate of glycolysis. The product of src may also catalyze phosphorylation of phosphatidylinositol mono-and biphosphate.
4. Diacyl glycerol stimulate the activity of the plasma membrane-bound protein kinease C which in turn phosphorylase a number of proteins some of which may be components of iron pumps.
5. Mild alkalinization of the cell brought about by activation of an Na\(^+\)/H\(^+\) antiport system can play a role in stimulating mitosis.
6. The product of src may, therefore, affect a large number of cellular processes by its ability to phosphorylate various target proteins and enzymes and by stimulating the pathway of synthesis of the polyphosphoinositides.

Oncogenes of other Retroviruses:

1. About 20 oncogenes of other retroviruses have been identified. Almost half of the products are protein kinases, mostly of the tyrosine type.
2. Some of these encode protein kinases & remaining encode various other proteins.
3. The product of the ras oncogenes of murine sarcoma viruses binds GTP, has GTPase activity and is related to the proteins that regulate the activity of the important plasma membrane enzyme, adenylate cyclase.

**Activation of Protooncogenes to Oncogenes:**

Following steps take place in this activation:

1. Promoter Insertion
2. Enhancer Insertion
3. Chromosomal Translocations
4. Gene Amplification
5. Single point Mutation

**II.3 Biological Carcinogens—infections from certain viruses, bacteria or parasites:**

Limitations to study chemistry of cancer – information about how cell growth – both normal or pathologic is controlled, is limited\(^{(1,7)}\). Knowledge of specific genes involved in regulation is even more meager. Little is known about biochemical basis of metastasis. Lastly the molecular basis of differentiation is little known. There has been recent surge of interest in elucidating the molecular basis of genetic susceptibility to cancer eg. Isolation of BRCA-1 gene which increases susceptibility to breast and ovarian cancer.

**Cancer cells are characterized by three properties:**

1. Diminished or unrestrained control of growth.
2. Invasion of local tissues and
3. Spread or metastasis to other parts of the body.

Cells of Benign tumours also show diminished control of growth but do not invade local tissues or spread to other parts of the body.
Characteristics of Cancer cells:

Characteristics of Cancer cells are well defined.\textsuperscript{(1,7,11)}

A) Morphological Changes

- Cells usually are rounded shape larger than normal cells.
- Cells show nuclear and cellular pleomorphism, hyperchromatising, altered Nuclear: Cytoplasmic ratio, abundant mitosis, sometimes tumor giant cells.
- Transformed cells often grow over one another and form multilayer’s (Microtumours).
- Cells can grow without attachment to the surface in vitro i.e. diminished adhesion.

B) Biochemical Changes

- Increased synthesis of DNA and RNA.
- Increased rate of glycolysis, both aerobic and anaerobic.
- Alternations in permeability and surface charge.
- Changes in composition of glycoproteins and glucosphingolipids on cell surface.
- Alterations of the oligosaccharide chains.
- An increased activity of ribonucleotide reductase and decreased catabolism of pyrimidines.
- Secretion of certain proteases and protein kinases.
- Alternations of isoenzyme patterns often to a foetal pattern and synthesis of foetal proteins, eg. carcinoembryonic antigen (CEA), α fetoprotein (AFP) etc.
- Appearance of new antigens and loss of certain antigens.

There is an inappropriate synthesis of certain hormones and growth factors – often there may be increased secretion of certain growth factors into the surrounding medium.
Role of DNA:

DNA is a critical macromolecule in carcinogenesis and is the premier target molecule in carcinogenesis which is being established\(^7\) by following facts:

1. Cancer cells beget cancer cells i.e. essential changes responsible for cancer are transmitted from mother to daughter cells. This is consistent with behavior of DNA.
2. Both, irradiation and chemical carcinogen damage DNA and are capable of causing mutations in DNA.
3. Many tumour cells exhibit abnormal chromosomes.
4. Transfection experiments indicate that purified DNA (Oncogenes) from cancer cells can transform normal cells into cancer cells (potential)
5. Genes that increase susceptibility to cancer have been isolated. However (methylation of DNA) epigenetic factors may also play role in carcinogenesis.

Types of Cancer as per the Modern Terminology:

Cancer is a complex set of diseases. Each cancer is unique in the way it grows and develops, its chances of spreading, the way it affects one’s body and the symptoms one may experience. Several factors, including location and how the cancerous cells appear under the microscope, determine, how cancer is diagnosed. All cancers, however, fall into one of our broad categories as described below\(^3\).

**Carcinoma**: Carcinoma is a malignant neoplasm of epithelial origin i.e. a tumor of epithelial origin It is a tumor that arises in the tissues that line the body’s organs like the nose, the colon, the penis, breasts, prostate, urinary bladder and the ureter. About 80% of all Cancer cases are Carcinomas.
Sarcoma: Sarcomas are tumors that originate in bone, muscle, cartilage and fibrous tissue or fat. Ewing sarcoma [family of tumors] and Kaposi’s sarcoma are the common types of sarcomas.

Leukemia: Leukemia’s are cancers of the blood or blood-forming organs. When leukemia develops, the body produces a large number of abnormal blood cells. In most type of Leukemia, the abnormal cells are white blood cells.

Lymphoma: Lymphoma affects the lymphatic system, a network of vessels and nodes that acts as the body’s filter. The lymphatic system distributes nutrients to blood and tissue and prevents bacteria and other foreign 'invaders' from entering the blood stream. There are over 20 types of lymphoma, Hodgkin's disease is one type of lymphoma. All other lymphoma’s are grouped together and are called non-Hodgkin's lymphoma.

Concept of Cancer in Ayurveda:

Ayurveda considers Cancer an emotionally caused disease. According to Ayurveda, either less or excess or untimely diet or lifestyle factors that disturb Vata Pittakapha can cause cancer. Rasayana Shastra, the clinical specialty of Ayurveda emphasizes the balance of seven metals (gold, silver, copper, tin, zinc, iron and lead) which are considered to help in prevention and treatment of many diseases. The food items that we consume today are deficient in many minerals. This deficiency causes acidification which further leads to poor absorption of minerals. Moreover, eating disorders, untimely eating, exertion, anxiety, stress, depression and other lifestyle factors result in undesirable effects such as inflammation of the gastro-intestinal tract. Accumulation of toxins and the gradual suppression of the immune system lead to abnormal reaction in different tissues of the body, ultimately leading to cancer.

Thus Ayurveda considers Aahaar and Vihaar as one of the main etiological factors for precipitation of cancer. There are certain types of
diets which modify the intestinal flora. Consumption of smoked fish and rice play an important role in the development of carcinoma of stomach. Low residual and high protein diets are considered to be primary cause of large bowel carcinoma. Similarly, Cigarette smoking may lead to carcinoma of bronchus. Use of betel with lime and tobacco or excessive use of tobacco precipitates oral cancer (3).

**Tumour Immunology:**

All forms of treatment of cancer (surgery, radiotherapy and chemotherapy) leave some residual cancer cells in the body. These are annihilated by the body's immune mechanism. All the effect arms of immunological mechanisms are active against cancer cells. These are

1. T-cells
2. NK cells
3. Antibody dependant complement mediated lysis
4. Antibody dependent cell mediated cytolysis (ADCC) and
5. Macrophages

**Tumour markers** are defined as a biochemical substance (eg. hormones, enzymes or proteins) synthesized and released by cancer cells or produced by the host in response to cancerous substance and are used to monitor or identify the presence of a cancerous growth. The term “tumour marker” designates a broad category of substances produced by malignant cells or by benign cells in response to the presence of malignancy. They may be applied to the detection, identification, monitoring, radio-localization and therapy of malignancies.

Sites :- Tumour markers may be present

- In blood circulation
- In body cavity fluids
- In cell membranes
- In cell cytoplasm
The tumour markers are different from substances produced by normal cells, in quantity and quality.

**Diagnosis:**

1. Immunohistological and immunocytological tests are used to detect those tumours markers which are present only on cell-membranes and cytoplasm of cells and not in blood circulation\(^1\). Examples are as follows.
   - Immunofluorescence
   - Immunoperoxidase
   - Monoclonal antibody technique

2. Biochemical methods are used for measuring tumour markers found in the blood circulation. Examples are as follows.
   - Radioimmunoassay RIA
   - Enzyme-immunoassay
   - Immunochemical reactions

**Clinical Uses of Biochemical Tumour Markers:**

Ideally tumour markers have following six potential uses in cancer patient care\(^1\). They are -

1. For screening, especially in asymptomatic population.
2. For diagnosis in asymptomatic patient.
3. As a prognostic predictor.
4. As an adjunct in clinical staging of the cancerous conditions.
5. For monitoring during treatment of the patients.
6. For early detection of relapse or recurrence of the cancerous process.

Tumour markers or Tumour index substances are factors released from the tumour cells which could be detected in blood and therefore
indicate the presence of the tumour in the body. They are also useful for the following purposes

1. For follow up of cancer and to monitor the effectiveness of the therapy and also to detect the recurrence of the body tumour.
2. To facilitate detection of cancer: The presence of tumour markers suggests the diagnosis, but caution is to be taken to rule out other nonmalignant conditions.
3. For prognosis: serum level of the marker may indicate roughly the tumour load and which in turn indicates whether the disease is curable or not.
4. For localization: Experimentally it is shown that radio labeled antibodies against the marker will be fixed on the tissues producing the marker.
Table 1.4

Some Clinically useful tumour markers are listed in the following Table

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviations</th>
<th>Serum level is increased in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncofetal Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Alpha fetoprotein</td>
<td>AFP</td>
<td>Hepatoma, Germcells Cancers</td>
</tr>
<tr>
<td>▪ Carcinoembryonic antigen</td>
<td>CEA</td>
<td>Colorectal, Gastrointestinal, lung cancer</td>
</tr>
<tr>
<td>▪ Ovarian cancer antigen</td>
<td>CA-125</td>
<td>Ovarian Cancer of epithelial origin</td>
</tr>
<tr>
<td>▪ Beta oncofetal antigen</td>
<td>BOA</td>
<td>Pancreatic Cancer</td>
</tr>
<tr>
<td>▪ Tumour antigen</td>
<td>T-24</td>
<td>Bladder Cancer</td>
</tr>
<tr>
<td>Enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Alkaline Phosphatase</td>
<td>ALP</td>
<td>Bone Secondaries</td>
</tr>
<tr>
<td>▪ Placental alkaline phosphatase</td>
<td>Regan</td>
<td>Lung, Testicular seminoma</td>
</tr>
<tr>
<td>▪ Prostatic acid phosphatase</td>
<td>PAP</td>
<td>Prostate Cancer</td>
</tr>
<tr>
<td>▪ Neuron Specific Enolase</td>
<td>NSE</td>
<td>Nervous System Tumours</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Beta chain of Human Chorionic gonadotropin HCG</td>
<td>Beta-HCG</td>
<td>Choriocarcinoma</td>
</tr>
<tr>
<td>▪ Calcitonin</td>
<td></td>
<td>Medullary thyroid carcinoma</td>
</tr>
<tr>
<td>▪ Big ACTH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Adapted from reference No. 1]
Metastases:

Metastasis is the spread of cancer cells from the primary site of origin to other tissues, both neighboring and distant, where they grow as the secondary tumours\(^{(1,7,10)}\). When cells break away from a cancerous tumour they can travel through the bloodstream to other areas of the body. They can end up in any organ or tissue. Cancer cells can also travel through the lymph system. This system includes lymph nodes (small, bean-sized collections of immune cells), which are connected by lymph vessels. The lymph vessels are much like blood vessels, except they carry a clear fluid called lymph back towards the heart. Cancer cells that travel through the lymph system often end up in the lymph nodes, but they can also spread to other organs.

Many of the cancer cells that break off from the original tumour die without causing any problems. But some settle in a new area. There, they begin to grow and form new tumour. This spread of cancer to a new part of the body is called Metastasis. It is complex process to analyze in humans. It is the spread of cancer cells from a primary site of origin to other tissues where they grow as secondary to tumours, it is the major problem presented by the disease. Loss of anchorage dependence, diminished contact inhibition and increased cell surface charge are contributory factors for the tendency of cancer cells to disintegrate from the main mass and to dessaminate to nearly or distant organs. This forms Metastasis\(^{\text{(11)}}\).

Sometimes metastatic tumours are found by tests that are done when the primary cancer is first diagnosed. In other cases, the metastasis is found first, causing the doctor to look for the place that the cancer started. Sometimes, no metastasis are seen when the cancer is first found. Instead, they are found later, the patient has been treated and was thought to be cancer free. When a cancer spread to bone, in people with breast and prostate cancer, the bone is often the first distant site where the cancer spreads. As seen further in our research work this correlates with the
parameter serum alkaline phosphatase showing increased levels where bone involvement is seen \(^{(11)}\).

As described earlier, the word CANCER is derived from a Greek word meaning CRAB. The cells from the main cancer tissue migrate faster away, like the feet of a crab. The collagenase and stomolysin released by most cancers help in the penetration of cancer cells into surrounding areas. Retinoic acid is shown to decrease collagenase and stromolysin enzymes produced by cancer cells, this may explain the anticancer activity of vitamin ‘A’ which is studied further as an antioxidant parameter.

The malignancy of tumour cell tends to progress –

1. When a cell becomes a tumour cell, the composition and behavior of the cell do not remain static. Rather tendency for malignancy is increased. This is manifested by increasing rates of growth, and an increasing tendency to invade and metastasize.

2. The mutations in DNA repair genes are found in this phenomenon creating a mutator phenotype. The activation of additional oncogenes occurs. Cells are found with faster rates of growth.

3. The biochemical profile of highly malignant cells may be very different from that of normal cells. Many changes in enzyme profile also occur.

4. The fast growing cells tend to maximize the anabolic processes involved in growth, cut down on catabolic functions i.e. they are concentrating mostly on growth.

5. They also show biochemical changes reflect altered gene regulation, such as the synthesis of certain fetal proteins and the manufacture of hormones \(^{(13)}\).
Fig. 1.5

DIAGRAM OF SCHEMATIC COMPENDIUM\textsuperscript{(1,12)}

Certain Enzymes and Proteins

Released

Cancer Cells

Oncogenic Virus

Focused

CANCER

ONCOGENES

Focused

For treatment

Growth Factors

Chemical Agents

Damaged or Altered DNA

Anticancer drugs

Biologic Agents

Physical Agents

P. Glycoprotein

Resistance
Proceeding of Metastasis:

1. Benign tumours can grow very rapidly and attain big sizes and may be sometimes life threatening but they do not metastasize.

2. It is the malignant tumours, cancerous ones invade surrounding tissues and send out cells to begin new tumours at distant sites. The spread may be blood borne or through lymphatics.

3. This colonization at distant sites is metastasis and is the major cause of death from human malignancies.

4. Tumours cells must attach to, degrade and penetrate the “extracellular matrix” (ECM) at several steps of metastasis. Thus metastasis biochemically is multistep process.

5. Approximately 50% of patients who develop malignant tumours can be cured with various therapies, viz surgical removal, radiation therapy and chemotherapy. Of the remaining 50%, majority die because of metastasis hence, in a real sense, if metastasis could be controlled, cancer could be controlled and for the most part, cured.

6. Current consensus opinion is that metastasis is an “active process of invasion” (1,12).

Metastasis has been shown to require:

- Specific surface receptors.
- Requires enzymes.
- The process uses energy required for protein synthesis.

This active process, metastasis, involves

- A metastatic cell has to penetrate the extracellular matrix (ECM) that surrounds the tumour.
- It travels to through the tissue till it reaches a blood vessel or a lymphatic.
• In case of blood borne metastasis, the tumour cell then attaches the blood vessel wall, dissolves a portion of the wall and propels itself through into the circulating blood.

• Metastatic cells often travel in the circulation as small clumps of cells, called “emboli”

• At a distant site, the tumour cell again reattaches to the blood vessel wall and repeats the process, traveling as much as two or three cell diameters into the invaded tissue before it settles down and begins to form a new tumour.

Composition of Extra Cellular Matrix (ECM) can be divided into two major categories –

- Basement Membrane (BM)
- Interstitial Connective Tissue (ICT)

**Interaction with basement membrane:**

BM is the first tough elastic barrier that surrounds both tissues and blood vessels. Hence an invading cancer cell must past this barrier several times, in order to establish metastatic colonies in distant tissues.

**Stages:**

The above interaction of cancer cell with Basement Membrane can be considered arbitrarily under 3 steps:

I. **Step 1:**
Attachment of the invading metastatic cell to basement membrane (BM)

II. **Step 2:**
Dissolution of the basement membrane, so that the cell can pass through it.

III. **Step 3:**
Migration of tumour cells - It has been shown that specific biochemicals are required for the tumour cells to complete the process. \(^{(14)}\)
Fig. 1.6

Flowchart depicting a simplified scheme of the molecular basis of Cancer\(^{(12)}\).

Acquired (Environmental) DNA Damaging agents
- Chemical
- Radiation
- Viruses

Normal Cell

Successful DNA repair

DNA Damage

Failure of DNA repair

Inherited Mutations in genes affecting DNA repair
- genes affecting Cell growth or apoptosis

Mutations in the genome of somatic cells

Activation of growth – promoting onco genes

Alterations of genes that regulate apoptosis

Inactivation of cancer suppressor genes

Expression of altered gene products and loss of regulatory gene products

Clonal expansion

Additional mutations (Progression)

Heterogeneity

Malignant neoplasm
Genes that regulate apoptosis:
- New category of genes that regulate programmed cell death is discovered. These are important in carcinogenesis.
- bcl$_2$ causes β cell lymphoma by preventing apoptosis it allows other mutations of proto oncogenes that ultimately lead to cancers.

Unified hypothesis of Carcinogens:
- Multifactorial origin of cancer is suitably explained on basis of oncogenes.
- The physical and chemical agents / viruses and mutation all lead to activation of oncogenes causing carcinogenesis.
- Antioncogenes and genes regulating apoprotein are intimately involved in development of cancer.

Figure 1.7
Hypothesis for development of Cancer$^{(1,7)}$

Environmental factors
(Physical and Chemical)
Types of Biomarkers:

The biomarkers used today in medicine and research generally fall into several categories. Molecular biomarkers, also called molecular markers or biochemical markers, are one of the most common types. Broadly these biomarkers are described under following three categories:

1. Molecular or biochemical biomarkers –
   These are biological molecules found in body fluids or tissues. In cancer, molecular biomarkers are often genes or gene products such as proteins, molecular biomarkers are no longer confined to a single molecule. Instead, they may consist of a panel of different biochemical entities that together serve as a biomarker signature.

2. Physiological biomarkers –
   Physiological biomarkers are those that have to do with the functional process in the body. For instance blood flow in brain areas affected by stroke is being investigated as a potential indicator of treatment success.

3. Anatomic Biomarkers –
   Anatomic biomarkers are those that have to do with the structure of an organism and the relation of its parts. Anatomic biomarkers include the structure of various organs such as the brain or liver.

Significance of biomarkers:

Most of the definitions note that biomarkers may have at least one of several purposes:

(i) to help diagnose a condition, perhaps before the cancer is detectable by conventional methods; this is known as a diagnostic biomarker.

(ii) to forecast how aggressive the disease process is and / or how a patient can expect to fare in the absence of therapy; this is known as a prognostic biomarker, and

(iii) to help identify which patient will respond to which drug; this is known as a predictive biomarker.
Table 1.5
Biomarkers

<table>
<thead>
<tr>
<th>Role of Biomarker</th>
<th>Use</th>
</tr>
</thead>
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<tr>
<td>Diagnostic</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Predictive</td>
<td>To help identify which patients will respond to which drugs.</td>
</tr>
</tbody>
</table>

Tumour Markers:

**Definition:** The National Cancer Institute defines a tumour marker as “a substance that may be found in tumour tissue or released from a tumour into the blood or other body fluids”. The phrase tumour marker is often used interchangeably with biomarker. However, the definition of biomarker is broader. Biomarkers include not only substances associated with or released from tumour tissue, but also physiological markers or markers visualized using imaging technology. Biomarkers may also be substances released by the body in response to the tumour but not by the tumour itself. For instance, the immune system may react to the tumour by producing substances that can be detected in the blood. These substances may indicate the presence of a tumour, but are not actually produced by the tumour cells. Additionally the term biomarkers can apply to blood cancer, which do not form solid tumours (8).
Clinical Orientation:

- After cardiovascular disease, cancer is the second common cause of death in world.
- Humans of all ages are victimized by cancer and different organs are affected by it.
- Many cancers are related to the increase of age: the development of this disease is associated with the longer life.
- Proper treatment by surgery, radiotherapy and chemotherapeutic agents in early stages of the disease can control the spreading of it. Otherwise metastasis can develop rapidly with acute and incurable symptoms causing death in the long run.

Lipid Peroxidation and Cancer

Lipid peroxidation is an oxidative chain reaction in which one lipid molecule after another becomes oxidized to the maximum possible extent or so as to form a lipid peroxide [i.e., a lipid molecule containing one or more O–O bonds](15). Most biological membranes are extended bilayers of amphiphilic lipids with hydrophobic moieties directed to the center and hydrophilic head groups at the two surfaces. Biological cell membranes are packed with polyunsaturated fatty acids (PUFAs), such as arachidonic and docosahexaenoic acid, in either the isolated form or the incorporated form in triacylglycerides and phospholipids. PUFAs are particularly susceptible to peroxidation. With increasing concerns about the potential adverse effects of lipid peroxidation in cellular membranes, the relevance of lipid peroxidation to biology and human diseases has been extensively explored since the 1950s.

Mechanism of Lipid Peroxidation

Lipid peroxidation is a free radical-initiated chain oxidation of lipids. Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of two electrons. A free radical is defined as
any chemical species possessing one or more unpaired electrons and capable of independent existence. Hydroxyl (OH) and superoxide (O$_2^-$) are examples of oxygen-centered radicals. There are also other types of radicals such as thiol (RS) trichloromethyl (CCl$_3$), and nitric oxide (NO) A free radical is marked by a dot, which designates the presence of one or more unpaired electrons. Lipid peroxidation can be divided into three separate

**Processes** – initiation, propagation, and termination. During initiation a very small number of Radicals (e.g., transition metal ions or a radical generated by photolysis or high-energy irradiation) can abstract hydrogen from lipid molecules to yield free radicals of lipids.

\[
X + RH \rightarrow R + XH
\]

Propagation then allows a reaction with molecular oxygen to form lipid peroxyl radicals.

\[
R + O_2 \rightarrow ROO
\]

This peroxide radical can then react with the original substrate, yielding one hydroperoxide and one new radical.

\[
ROO^- + RH \rightarrow ROOH
\]

Thus, the events form the basis of a chain-reaction process. The lipid hydroperoxide decomposition produces more radicals and noxious aldehydes.

\[
ROOH \rightarrow RO, ROO^- \text{aldehydes}
\]

When the substrate is depleted, termination reaction begins. Two radicals combine the unpaired electrons to form a nonradical product.

\[
R + R \rightarrow R-R
\]

The chain reactions are also terminated when antioxidants (A–H), which provide easily donatable hydrogen for abstraction by peroxyl radicals, combine.
Membrane products of lipid peroxidation are formed in transformations called β-elimination, which consists of breakage of the bonds in β-position in relation to the unpaired electron. Polyunsaturated fatty acids undergo disintegration yielding toxic aldehydes, hydroxyaldehydes, and hydroxyalkenales. End products of lipid biodegradation, such as malondialdehyde, produce colored products with thiobarbituric acid (with maximum absorbance at 535 nm) in the principle of the most often used colorimetric method in lipid peroxidation studies (15).

Oxidative Stress and Antioxidants:

Cellular oxidative damage is a cell established general mechanism of cell and tissue injury that is primarily caused by free radicals FR and reactive oxygen species (ROS). Low levels of ROS are indispensable in many biochemical processes (25) however, overproduction and/or inadequate removal of ROS can result in oxidative stress, which is characterised as an imbalance between the formation of active oxygen metabolites and the rate at which they are scavenged by enzymatic and non-enzymatic antioxidants (AO). Oxidative stress can participate in the pathogenesis and complications of many disease including cancer (16).

The oxidative stress to genetic material has been proposed to be initiating step in diseases like cancer, ageing and autoimmune disorders (17).

Antioxidants (AO) have gone through a gradual transition from “miracle molecules” to “marvellous molecules” to “physiological molecules” (18-37). They are vital in numerous metabolic reactions and are co-players in redox homeostasis (38-42).

The involvement of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in human physiology (25,27,41,43-51) results in free radicals (FR). FR generation is involved in etiopathogenesis of human
The raised oxidative stress (OS) in majority of disease is consequence and not the cause. An imbalance between free radical generations and sequestration leads to oxidative stress (OS). Raised OS is not present in all the patients and a specific level of OS is necessary for vital activities. Attempts to manipulate OS below this level may result in serious consequences.

Free radical created many reactive toxic non-radical species in track in the body before being finally brought down to ground state energy level molecule. These FR and their non-radical reactive species were derived from oxygen and nitrogen and were collectively designated as ROS and RNS. Since FR were accused as casual factors in a large number of disease these were referred as “Free Radical Disease”. Some of the important disease and health issues in this category are cancer, cardiovascular diseases, atherosclerosis, neurological disorder, renal, liver, hypertension, rheumatoid arthritis, adult respiratory disease syndrome, autoimmune deficiency diseases, diabetes mellitus etc.

Oxidative Stress has casual relationship in several pathological conditions such as insulin resistance and chronic diseases and AO can abolish or prevent these conditions. Excess generation of FR may not be causing the disease but vice versa i.e. the disease process may be aggravating the radical generation resulting in the raised OS. The raised OS in many instances could possibly be due to increased metabolic activity in disease process. In general antioxidants provide a preventive measure against the hazards of oxidative stress with their ability to neutralize, balance and sponge up free radicals by coupling with unpaired electrons.

**Antioxidants**

All the endogenous and exogenous compounds showing the free radical scavenging effect and possessing AO property were collectively termed as Antioxidants.
AO are defined as any substance that when present in low concentration compared to any oxidizable substrate (in biological system, everything except water) significantly delays or prevents oxidation of substrates.

**Broadly antioxidants are categorized in two groups.**

1. Enzymic Antioxidant
2. Nonenzymic Antioxidant

**The later are classified into two –**

A) Endogenous-eg. α-lipoic acid and reduced glutathione (GSH)
B) Exogenous (dietary)-eg. vitamins C, E and β-carotene.

AO must have following ideal merits –

1. Should have general property to quench all types of FR or specific quenching property.
2. Nullify or retard the action of redox active non-radical species.
3. Chelate redox metals, especially iron and copper.
4. Easily absorbed and distributed in tissues.
5. Should be tissue specific.
6. Should effectively enter in the proper slot of the AO network as electron flows from higher energy level to low energy level only.
7. Must be preferably soluble in both water and lipid medium which will make it equally effective in cytosol as well as membrane domains.
8. It should have the capability to repair damaged molecules eg. GSH repairs DNA damage and it should destroy the acutely damaged molecules and replace them with new ones. \(^{(88)}\)

Dong Wang *et al* \(^{(89)}\) studied total oxidant / antioxidant status in sera of patients with thyroid cancers. Oxidative stress is considered to be involved in the pathophysiology of all cancers. In this study Dong Wang *et al* evaluated the total oxidant / antioxidant status in patients with thyroid
They determined oxidative status, total antioxidant status (TAS) and total oxidant status (TOS) and calculated the oxidative stress index (OSI). Their findings suggested that oxidants are increased and antioxidants are decreased in patients with thyroid cancer. The OSI may be a more useful oxidative stress biomarker for monitoring the clinical status of thyroid cancer patients. Our study was in accordance with previous studies showing decreased AO levels in various clinical forms of malignancies.

Erdamar H studied that thyroid hormones regulate oxidative metabolism and thus play an important role in free radical production. Thyroid hormones regulate the synthesis and degradation of enzymes such as SOD, catalase, glutathione peroxidase (GPX) and glutathione reductase (GSH) and non enzymatic antioxidants such as Vitamin E and C, glutathione, uric acid, ferritin, transferin and ceruloplasmin. The changes in these enzymes and non-enzymatic substances affect the redox balance in the body and in turn, enzymatic feedback regulates thyroid function. The major effect of thyroid hormones is to increase mitochondrial respiration which results in upregulation of ROS, leading oxidative damage to membrane lipids.

Oxidative stress in many instances could possibly be due to increased metabolic activity in the disease process representing the phenomenon of “smoke after fire” rather than “inflicting effects of fire”.

Erythrocytes are a convenient and frequently used model for studies of cellular oxidative stress due to their availability, relative structural simplicity and quite well established functions. Erythrocyte membrane contains high levels of polyunsaturated fatty acids due to which these cells are continuously exposed to oxygen, erythrocytes show marked resistance to oxidative damage.
K. Kolanjiappan et al (92) aimed to examine the structural integrity of red blood cells in cervical cancer patients by measuring the concentration of TBARS, antioxidant status, cholesterol / phospholipid (C/P) molar ratio, enzyme activity and osmotic fragility of erythrocytes (SEPH). The red cell fluidity and permeability were determined by estimating the C/P ratio and Na⁺K⁺-ATPase activity, respectively. The results showed that the release of thiobarbituric acid reactive substances was significantly higher in cervical cancer patients as compared to normal subjects. There was increased lipid peroxidation with concomitant decrease in antioxidants. The red blood cells of cervical cancer patients were more fragile than those from normal subjects. They concluded that increased lipid peroxidation, insufficient antioxidant potential and changes in C/P molar ratio and activity of Na⁺K⁺-ATPase cause structural and functional abnormalities in the erythrocytes of cervical cancer patients. (92)

Free radicals are extremely reactive, which is due to their tendency to attain stability by pairing their unpaired electron. For this reason, under certain conditions, free radicals can capture electrons from such biological molecules such as proteins, nucleic acids, and lipids. The latter are particularly sensitive to the free radical attack because they are components of cell membranes, and the loss of an electron triggers a free radical chain reaction, in which fatty acids are oxidized to peroxides, which is called lipid peroxidation. Final products of lipid degradation, such as malondialdehyde (MDA) and others, yield colored products after reaction with thiobarbituric acid.

Gupta S et al studied assessment of oxidative stress and effect of antioxidant supplementation during radiotherapy in carcinoma of upper digestive tract. Oxidative stress was studied by estimating plasma levels of MDA, beta carotene, vitamin E and Erythrocytic superoxide dismutase (E-SOD) activity in 50 cases of carcinoma upper digestive tract which included carcinoma of oral cavity, pharynx and oesophagus. Group A
patients (supplemented with antioxidants) showed significant elevation in beta carotene than group B patients (without antioxidant supplementation) thus showing beneficial effect of administration of antioxidants during radiotherapy without disturbing the therapeutic effect of radiotherapy.\(^{(93)}\)

**Role of Antioxidants:**

- **Vitamin A**

Vitamin A is known to be necessary for embryonic development precisely because it helps to 'differentiate' stem cells, pushing them to become required tissue. There is an anti-cancer drug that specifically acts by blocking the breakdown of retinoic acid, derived from vitamin A. This approach has been found to be surprisingly effective in treating animal models of human prostate cancer.

  A vitamin A derivative "cloud protect against lung cancer development in former smokers"\(^{(94)}\), significantly, the vitamin A derivative is used combined with alpha-tocopherol (Vitamin E) in order to reduce toxicity known to be associated with 13-cis-RS (the vitamin A derivative) therapy."

  A study published in the Journal of Nutritional Biochemistry found that administering both vitamin A and vitamin C to cultured human breast cancer cells was more than three times as effective as the administration of either compound, alone (since) the combination of the two vitamin inhibited proliferation by 75.7 percent compared to untreated cells. The ability of retinoic acid (vitamin A) to inhibit tumour cell proliferation is well known, although its mechanism is not been defined\(^{(94)}\). The authors suggest that the synergic effect observed in this study is due to ascorbic acid's ability to slow the degradation of retinoic acid therapy increasing vitamin A's cell proliferation inhibitory effects. Dietary intake studies suggest an association between diets rich in beta-carotene and vitamin A and a lower risk of many types of cancer. A higher intake of green and
yellow vegetables or other foods sources of beta carotene are suggested to prefer.\(^{(94)}\)

**The Glutathione-Cancer Connection:**

Glutathione is the most important antioxidant in the body. The immune system cannot function properly without it and antioxidant such as vitamin C and E rely on it to function properly within the body. Mitochondria, the major source of energy in the cells, would burn up without the presence of glutathione. Most importantly however, it is required to fight off disease and act as an immune system boosters. While the reasons for it are not entirely known, the glutathione and cancer connection has been well established. Patients with cancer, serious chronic illness, AIDS and over 60 other diseases have reduced glutathione levels.

"No other antioxidant is as important to overall health as glutathione. It is the regenerator of immune cells and the most valuable detoxifying agent in the body. Low levels are associated with early aging and even death".\(^{(95)}\)

**Aims and Objectives**

The present study deals with the evaluation of biochemical parameters in various malignancies. The results were compared with healthy control subjects. The malignancies studied were that of breast, cervix, and ovarian tumours (Chapter III) and that of oral and oral related cancers (Chapter IV).

The following serum / blood parameters were determined in the malignancies under study, in view of assessing the correlation among them and further with the treatment and therapeutic means of the disease (chapter III and IV).
1. **Kidney Function Tests** :
   - Blood Urea
   - Serum Creatinine
   - Serum Uric Acid

2. **Liver Function Tests** :
   - Serum Bilirubin
   - Serum Alkaline Phosphatase
   - Serum Aspartate Transaminase [AST/SGOT]
   - Serum Alanine transaminase (ALT/SGPT)
   - Serum Total Proteins
   - Serum Albumin

3. **Oxidative Stress Biomarkers** :
   - Lipid peroxidation : Malondialdehyde (MDA) or Thiobarbituric Acid Reactive Substances (TBARS)
   - Sensitivity of Erythrocytes to Peroxide Haemolysis (SEPH)

4. **Serum Antioxidant Levels** :
   - Vitamin A
   - Reduced glutathione (GSH)

The patients were divided into two groups viz the Test group and the Healthy i.e. Control group. For comparisons with test group study purpose, the healthy control subjects without any diseased condition were selected and treated as Control.

**The present study was undertaken with the following Aims and Objectives:**

1. To study the Kidney function tests (blood urea, serum creatinine and uric acid level) in patients having **breast cancer**, **cervix cancer**, **ovarian tumours**, and **Oral and oral related cancers** including
cancer of cervical nodes and secondaries in neck, pyriform fossa, maxilla, Parotid tumours, larynx, oesophagus.

2. To study the Liver function tests [Serum bilirubin, serum alkaline phosphatase, serum glutamate oxaloacetate transaminase (SGOT/AST), serum glutamate pyruvate transaminase (SGPT/ALT), serum total proteins and serum albumin levels] in patients having above said cancers.

3. To study the oxidative stress-lipid peroxidation (TBARS) and sensitivity of erythrocytes to peroxide haemolysis (SEPH) in the patients with above mentioned malignancies.

4. To study the serum antioxidant levels (Vitamin A and reduced glutathione) in patients having above said malignancies.

5. To study the correlation of the biochemical parameters with various malignancies [under study] at different malignant stages.

Subsequent chapter deals with the methodology used, (Chapter II) followed by the chapters presenting the results of the experiments/estimations conducted and the discussion related to the results (Chapter III and IV). Summary and overall conclusions are included in the last chapter (Chapter V).

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