CHAPTER - I

GENERAL INTRODUCTION
General Introduction:

Cancer research, or oncology, is not a science in itself but the simultaneous and frequently coordinated activity of many independent scientific disciplines, viz. clinical medicine, surgery, pathology, radiation physics, genetics, immunology, endocrinology and biochemistry. Cancer as a disease was recognized in antiquity and, in deed, its very name is derived from the ancient description of its frequent clinical course. With development of microscopic technique, the fine morphological details of tumor became evident to the pathologist, and the foundation of the scientific study of these kinds of abnormal growths was laid. The morphological description of tumors became not only of practical aid to the surgeon, but by classifying these lesions into various categories, was the necessary step in their understanding. The goal towards which all oncological disciplines are directed is not yet reached, although progress has undeniably been made. The mystery of cancer, and the human tragedy which it encompasses, have endowed it with an emotional quality and an urgency which open pitfalls to the unwary and innumerable disappointments to scientific and lay public alike (Greenstein, 1954). There is still much ground to be covered before the end is in sight, but science admits of no insuperable barricades.

Cancer is a very complex disease. It is a phenomenon of abnormal growth of cells. When cells in a part of the body begin to grow out of control is generally known as cancer. During the early years of an organism's life, normal cells divide more rapidly until the organism becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries. But the cancer cells are different from normal cells. They continue to grow and divide and instead of dying the cancer cells outlive normal cells and continue to form new abnormal cells. The developing cancer cells do not have any particular form and in the process of cell division they gradually lose their original structural pattern.

The Cancer develops due to malfunctioning of the DNA and it generally happens because of damage to DNA. Damage to the DNA occurs due to various
reasons like sudden or prolonged changes in the environment of the cells. Growing cancer cells usually forms tumors. But some cancers, like leukemia, do not form tumors. These cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow.

Cancer generally spread from one area of the body to the other areas. Cancer cells or the malignant DNA materials travel to other parts of the body through blood stream or lymphatic, where they begin to grow to new set of cancer tissue or tumour. This process is called metastasis. However, all tumors are not cancerous. There are some benign (noncancerous) tumors develop in the body and they do not spread (metastasize) to other parts of the body and are generally not life threatening.

The factors responsible for developing cancer can include a person's age, sex, and family medical history. Others are linked to cancer-causing factors in the environment. Still others are related to lifestyle choices such as personal habits like -betel nut and tobacco chewing, alcohol consumption and diet, exposure to sunlight and other environmental and occupational factors. These risk factors signify that a person is more likely to develop cancer at some point/stage in lives. However, having one or more risk factors does not necessarily mean that a person will get cancer. Some people with one or more risk factors never develop this disease, while other people who do develop cancer have no apparent risk factors.

The efforts to control the cancer is known since the dawn of civilization. In ancient India and Egypt vinegar and arsenic were applied to neoplastic lesions with hot iron directly or after cautery and therefore, modern approach to cancer prevention and control was not completely unrelated to the ancient physicians (Selkirk, 1980) Cancer control treatment measures are aimed at the interruption of cellular divisions of tumor. But due to the multifaceted etiology of cancer cells along with the inadequacy of knowledge of cellular biochemistry of cancerous cells, attempt on blocking the cancerous growth are often ineffective. For example clinical data show that a particular cancer preventive drug acts properly in a
patient while the same drug is found to be totally ineffective in another patient (Selkirk, 1980). It is therefore, relevant to understand the biochemical schemes within the growing cell that lead to the malignant state. Even in this age of scientific advancement the causation of disease cancer is still not fully understood and so control or cure of the disease is continue to be a great challenge for the mankind.

Due to the complexness of the disease, the cancer is emerging as a major public health problem in the present day world. Among all the types, high incidences of cancers of upper aero-digestive tract (UADT) have been reported from all the continents. Incidences of these cancers are four times higher in developing countries than that of the developed countries (Ramesh, 1993). In India also, especially in the northeastern part of the country, cancers of the upper aero-digestive tract are the commonest type of cancer occurring predominantly(NCRP, 2002).

Northeastern region of India has diverse population comprising many ethnic groups of people having different language, culture and life style. The dietary habits are also quite different from the other parts of the country. The region is covering an area of 2,55,028 sq. km sharing about 3.5% of the India's total population. The region comprises of seven states viz. Assam, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Meghalaya and Tripura. The region has international borders on three sides with China, Bhutan, Burma and Bangladesh. The topographical setup of the region is peculiar comprising of tough terrain with mountains, hills and valleys of different structures. Many areas specially the hilly districts are still lacking in communication for conveyance and are so underdeveloped. The state, Assam, has a diverse tribal and non-tribal mix of lifestyle and cultures. As per the NCRP (2002) the regions has been recorded with very high incidences of cancer of UADT. High incidence of the UADT cancer in the region has been attributed to some personal habits like chewing of Betel quid and tobacco along with other factors like general food habit, individual habit, geographical distribution pattern, age, sex etc (Sarmah, 1994)). Chewing betel quid and tobacco is a common habit in India and some of other parts of the South
East Asia. But in some northeastern states of India, specially Assam, use of Betel quid with tobacco is a tradition. Previous study reported that betel nut chewers with tobacco increased the risk of incidence of cancer (Jussawalla, 1981). However, no definite etiology of the disease has so far been established. Descriptive epidemiology of oral and pharyngeal cancer over the past four decades has already been reviewed, with specific focus on Europe and it was observed that the disease is highly related with the use of tobacco and alcohol along with low in fruit and vegetable diet (La Vecchia et al., 1997).

As definitive data for the region are still lacking on the subject, there is enormous significance of undertaking epidemiological investigation to study the geographical pathology of UADT cancer in the northeastern region and also to carry out basic studies to find out whether any correlation exists between the occurrence of the disease with the habits of chewing betel quid and tobacco.

Researchers have paid great efforts for cancer prevention and diagnosis. It has been found that there are certain relations between the substances in environmental exposure and cancer. Exposure to these substances over a certain level may induce the process of carcinogenesis. These toxic substances include various organic compounds and elements (Bernard el al., 1991). From the standing point of cancer prevention and diagnosis, the studies on the relationship between the cause of cancer and the levels of toxic substances have great significance.

During the past two decades, there has been a growing recognition that metal compounds are an important class of environmental and occupational carcinogens. Many studies, both epidemiological and experimental, had earlier reported about the possible carcinogenic role of metals such as arsenic, beryllium, cadmium, chromium, cobalt, lead, nickel, zinc, and iron (Seltzer et al., 1970; Anon., 1976; Sunderman, 1978, 1980; Heck and Costa, 1983). In some cases metals serve as constituents of vital biological molecules (eg. Iron in hemoglobin). Metals play an essential role as chelating agents (Schubert, 1981) and components of metalloenzymes (Riordan, 1976; Ulmer, 1977; Simkiss 1979).
Many reports exist in literature which confirm the distribution pattern of trace elements are very specific for certain types of malignancies. Trace elements, which occur as micro constituents of cell and tissues, may either enhance or retard the kinetics of anabolic or catabolic enzymes (Porise et al., 1979). It is increasingly evident that a relationship exists between the cancer and the levels of various elements in the human body (Porise et al., 1979).

Trace elements play important roles and are increasingly recognized as versatile anti-carcinogenic agents and several biological mechanism have been proposed to explain how the trace elements could reduce the incidence of a number of various cancers which involve the antioxidant potential of trace element dependent enzyme system (Koyama, 1996). Feldstein et al. (1998) determined a pronounced effect of Rb level in tumour by a factor ranging between 4 and 10 relative to normal tissue with a corresponding decrease in the Br/Rb level in C26 induced colon carcinoma and in induced 19 Balb mice using PIXE and XRF. Thus the importance of element concentration and their ratio value in diets as well as the tissues have been appeared to play significant role in understanding the cancer biology.

Numerous epidemiological studies have implicated arsenic, cadmium, chromium and nickel as human carcinogens, while compounds of beryllium, cadmium, cobalt, chromium, iron, nickel, lead, titanium and zinc have been used to induce cancers in experimental animals. Many of these studies have indicated that metal ions can interact with nucleic acids to influence base pairing and conformation. Such effects have been known to cause somatic mutations leading to cellular transformation. For example, Mg, Mn and Zn are co-factors of many enzymes especially RNA and DNA polymerase (Weinstein, 1978). It has been shown that a number of the carcinogenic metals cause infidelity in polymerase activity during DNA synthesis, whereas noncarcinogenic metals are refractory (Loeb et al., 1977). Andronikashvili et al. (1972) for the first time reported the variation of concentration of some heavy metals in nucleic acids in the process of neoplastic growth. Primarily zinc and secondarily some other trace elements are necessary for neoplastic growth in animals (Petering et al., 1967; De Wys et al.,
Andronikashvili et al. (1974) also concluded that DNA polymerase with its active zinc center might play an important role in the process of rapid DNA synthesis in malignant growth.

During the last two decades, many attempts have been made to investigate trace element concentrations in different biological tissues or fluids using various experimental techniques. A majority of the investigations have attempted to correlate measured data of trace element concentrations with the clinical stage of the examined disease as well as histopathological grading. The bulk of trace element investigations associate elemental concentrations with the study of tumour pathogenesis, course of disease and efficiency of medical therapy. Epidemiological studies have stressed the importance of nutrition, in particular vitamins (A,C,E), carotene, and trace elements as active compounds. The modulation of immune function by vitamins and trace elements remains important and affects survival.

Various techniques have been used to study the trace elements in biological samples and the popular among them are - particle induced X-ray emission (PIXE), X-ray fluorescence analysis (XRF) and its total reflection geometry method (TRXRF), atomic absorption spectrometry (AAS) and neutron activation analysis (NAA). Out of all these PIXE (particle induced X-ray emission) has been considered as the most advanced and sophisticated techniques widely used for study of trace elements in various types of samples(Zeynel, 1978).

Particle Induced X-Ray Emission Spectroscopy (PIXE) as an analytical tool:

To have a better understanding about the biological role of trace elements, the PIXE technique with the multi-elemental analysis and high sensitivity has been considered as a promising tool for evaluation of trace elements in life systems. The PIXE is now recognized as one of the most sophisticated tool for the detection and estimation of trace elements in the modern day research,
specially for the study of biomedical samples. It is a powerful yet non-destructive elemental analysis technique now used routinely in the study of biomedical subjects, life science, geological, archaeological samples etc.

The technique of particle induced X-ray emission analysis that has come to known as PIXE was introduced at the Lund Institute of Technology in 1970 for the first time in a brief exploratory publication which in retrospect may be seen as the starting point of a major and powerful technique of elemental analysis. It coupled two technical developments to create a novel variant of the general methods of X-ray emission analysis with unique capabilities.

The PIXE is based on a Quantum theory that states that orbiting electrons of an atom must occupy discrete energy levels in order to be stable. Bombardment with ions of sufficient energy (usually MeV protons) produced by an ion accelerator, will cause inner shell ionization of atoms in a specimen. Outer shell electrons drop down to replace inner shell vacancies, however only certain transitions are allowed. X-rays of a characteristic energy of the element are emitted. An energy dispersive detector is used to record and measure these x-rays and the intensities are then converted to elemental concentrations.

X-ray emission analysis involve both a means of exciting the atoms of a specimen with a proton beam so that they will emit characteristics X-rays and means of detecting and identifying these so that their intensities can be converted to elemental concentrations in the specimen.
PIXE System Description:

A finely collimated beam of protons is produced by a General Ionex tandem ion accelerator and brought through a graphite plate with a series of closely spaced 0.3 mm holes. An o-ring seals a thin polymer window over this plate providing a vacuum barrier through which the beam is brought. The graphite block provides mechanical support to the film and helps to conduct heat away from it greatly increasing its lifetime under beam bombardment. Relative beam...
current integration is measured off of the graphite block providing accurate normalization amongst samples. An internal Faraday cup provides secondary electron suppression. Because of this one can precisely measure and control the relative number of protons striking each sample. Accurate sample placement relative to that of the beam is accomplished by the aid of a HeNe laser beam which runs collinear to the ion beam. The sample is mounted on a block tilted as to provide the sample normal tilted 45 degrees from the incident ion beam. The sample area can be purged with He when looking for light elements with PIXE. A surface barrier detector is placed 60 degrees relative to the incident ion beam for RBS measurements at atmospheric pressure.

A Si(Li) x-ray detector is placed 90 degrees relative to the incident ion beam and is used to determine x-ray peak energies and intensities. X-ray absorber filters are used to attenuate the dominant peaks and allow greater trace element sensitivity. GUPIX, an interactive software package is used to analyze and convert raw spectral data into elemental concentrations (Johansson, 1995).

In India the PIXE facility is available only with the Department of Atomic Energy (DAE) of Govt. of India. This is a national facility offered by the DAE for carry out different line of research involving all the universities of in the country through an Inter University Consortium for DAE facilities. The facility is restricted because of its high operating cost and demand.

**Atomic Absorption Spectroscopy (AAS) as an analytical tool:**

Atomic Absorption Spectroscopy (AAS) is also widely used technique for determination and estimation of trace elements in biomedical studies, where only a single targeted element can be studied in one run. This tool is also used along with the PIXE technique for the study of specific elements. The normal procedure is to detect and estimate of all the trace elements present in the sample with PIXE.
technique and then deals the targeted elements specifically with the AAS technique, which is less expensive and readily available unlike PIXE.

Atomic-absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

Schematic diagram of an atomic-absorption spectrophotometric experiment:

The flame is arranged such that it is laterally long (usually 10cm) and not deep. The height of the flame must also be controlled by the flow of the fuel mixture. A beam of light is focused through this flame at its longest axis (the lateral axis) onto a detector. The light that is focused into the flame is produced by a hollow cathode lamp. Inside the lamp is a cylindrical metal cathode containing the metal for excitation, and an anode. When a high voltage is applied across the anode and cathode, the metal atoms in the cathode are excited into producing light with a certain emission spectra. The type of hollow cathode tube depends on the metal being analysed. For analysing the concentration of copper in an ore, a copper cathode tube is used, and likewise for any other metal being analysed. The electrons of the atoms in the flame can be promoted to higher orbitals for an instant by absorbing a set quantity of energy (a quantum). This amount of energy
is specific to a particular electron transition in a particular element. As the quantity of energy put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured (Smith, 1983).

The limitations of these technique is that this is destructive technique where the sample can be analysed for only once and can be useful for measuring some specific elements.

In the above described perspective the present study was undertaken to study about the high prevalence of cancer of UADT in the northeastern region of India and its probable causation. In this study both the above-described techniques PIXE and AAS are being used to detect and measure trace elements in cancerous tissues of human upper aero-digestive tract.
Bibliography:


** Originals are not seen.

End of the Chapter