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PROLOGUE
Cancer the dreadful disease, is one of the leading causes of morbidity and mortality worldwide. The prevalence of communicable diseases often hides the fact that cancer is also a serious problem in developing regions of the world. The major factors associated with high incidence of cancer include genetic and environmental factors. Exposure to environmental agents may be accidental, occupational or by lifestyle. It has been reported that about 70% of total cancers are related to lifestyle and nutritional factors all over the world (Reddy, 1988). Lifestyle elements include alcohol consumption and tobacco use through smoking and chewing. Epidemiological evidences indicate that use of tobacco is the most important established cause for cancers of lung and oral cavity. A clear-cut dose response relationship has been found between smoking and lung cancer as well as tobacco chewing and the cancer of the oral cavity (Blum A., 1993; IARC monographs, 1987; Ryan et al., 1992).

Cancer of the lung remains one of the leading causes of cancer deaths in adult men and recently has been established as the primary cause of cancer mortality in women also (Sagman et al., 1991). Cigarette smoking remains the greatest preventable cause of lung cancer mortality and it is responsible for 90% lung cancer deaths in American men and 75% American women (American Cancer Society, 1992). There is a strong evidence for a dose response effect of smoking, 24 fold excess risks have been observed for development of cancer among heavy smokers.
(Lerman et al., 1993). In developing countries like India, approximately 80-90% of all lung cancers are caused by tobacco smoking (Sanghavi, 1989). During last five years lung cancer constituted high incidence in India, and a large group of patients has been registered at our institute (The Gujarat Cancer and Research Institute, Ahmedabad) as well. As reported by our Cancer Registry Department (Biennial Report, 1988-89) lung cancer is the second most leading site in males and accounted for 12.3% of all cancers. The male to female ratio in lung cancer was 7.2:1. Because of insidious nature of the disease, a limited progress in management of patients with lung cancer has been observed. Only 20% of patients are diagnosed while the disease is localized. The five years survival for patients with localized disease is 27% and for those with regional metastases only 8% (Szabo et al., 1993).

Likewise, oral use of smokeless tobacco in various combinations with other ingredients like lime, areca nut etc. is predominantly associated with the causation of oral cavity cancer (IARC Monographs, 1987). More than 90% of tumours of the oral cavity among men and 60% among women are associated with use of smokeless tobacco (US Department of Health and Human Services, Surgeon General Report, 1989). Oral cavity cancer is known to be the most common in several Asian countries and in India these cancers account for almost 40% of total cancer deaths (Segi and Kurihara, 1972). Over all incidence of oral cavity cancer is higher than the incidence of most other cancers.
in India (National Cancer Registry: Annual Report, 1988). High incidence of the disease has also been reported by population based cancer registry and hospital based cancer registry of The Gujarat Cancer and Research Institute, Ahmedabad (Biennial Report, 1988-89). The cancer of the tongue was the first leading site among males which accounted for 12.4% of all cancers. The male to female ratio in tongue cancer was 7.3:1.

For lung carcinoma chest x-ray and sputum cytology are the major screening methods for detection of the disease, however these methods are not sensitive enough to detect lung cancer at an early stage. On the other hand, most of the lung cancers are disseminated at the time of presentation hence the management of patients with advanced lung cancer is difficult (Mulshine et al., 1993). Similarly deeply located oral cavity tumours may not be noted until grown to a large size and reached an advanced stage (Xing et al., 1991). All these aspects highly signify the need to pay attention towards early diagnosis and management of patients with cancers of lung and oral cavity. Unfortunately, none of the previously available biochemical markers have yet proved satisfactorily useful (i) to detect early disease in asymptomatic population (ii) to differentiate between malignant and non-malignant lesions (iii) to assess prognosis in cancer patients (iv) for treatment monitoring. More recently, attention has been drawn to the oncogenes that are believed to play a role in the development of cancer. A gene associated with the malignant state would clearly be a candidate for
production of general cancer associated substances. These substances are selectively released by tumour cells in serum or other body fluids and can be used as markers for clinical monitoring of various malignancies (Bates 1991; Consensus Report, 1987; Sell, 1993). Hence, the efforts are necessary to search for the simple, noninvasive and inexpensive blood-based diagnostic tests which can detect the cancer of lung and oral cavity at an early stage. It is essential to establish a biochemical marker system which can be helpful in differentiating patients with cancer and other non-malignant diseases and indicating disease status during follow up under anticancer treatment.

The normal cell, either spontaneously or under the influence of some physical or chemical agents may undergo biologic variations, which leads to an apparent failure of normal growth control mechanisms. These cells with aberrant biology may invade and disseminate in surrounding tissues. This uncontrolled growth is defined as 'malignant condition'. Many properties of living cells are expressed at or mediated through the cell membrane. In an organized tissue, the cell membrane governs interactions with cells of its own or different kinds. It also regulates the entry of nutrients, hormones, drugs and plays a decisive role in recognizing foreign proteins and thereby regulates a variety of phenotypic characteristics including growth, development and communications. Thus, cancer being a cellular disease, cell membrane changes including
qualitative and quantitative variations in glycoproteins, enzymes, glycolipids, lectin agglutinability etc. are of major interest in clinical oncology. Looking to the possible altered synthesis of these biomolecules by malignant cells search for clinical value of these substances as tumour markers has increased rapidly (Sell, 1993). A comprehensive diagram of the possible targets for changes in malignant cell is shown in figure-1.

Glycoproteins and glycolipids present in the membrane of transformed cell have altered carbohydrate composition compared to non-transformed progenitor cells. This may contribute to aberrant cell to cell recognition, cell adhesion, antigenicity and the invasiveness demonstrated by malignant cells (Hakomori, 1981; Hakomori, 1984; Yogeeswaran, 1983). Increased glycolytic activity has also been observed in cancer patients. Therefore, several glycolytic enzymes naturally assume importance as biological markers in diagnosis, prognosis and therapeutic monitoring of patients with various malignancies (Baumann et al., 1990; Damle et al., 1971b; Gomm et al., 1988; Santabarbara et al., 1988).

Recent reports from this laboratory (Patel et al., 1990a; 1990b; 1990c; 1990d; 1991; 1993b) and by various other investigators (Gosh et al., 1991; Kakari et al., 1991; Klapan et al., 1993; Oztokatli et al., 1991; Xing et al., 1991) indicate that glycoconjugates like total sialic acid, lipid bound sialic acid, fucose, seromucoid fraction (hexoses and mucoid proteins)
Figure-1

A comprehensive diagram of phenotypic changes in malignant cell
Lectin agglutination
Mobility of surface components
Inter-cellular communication
Adhesion
Phagocytosis
Permeability
Transport
Secretion
(enzymes, growth factors etc)

Receptors (growth factors, etc)
Tumour antigens
Fibronectin
Surface charge
Surface enzymes
Secretion (enzymes, growth factors etc)

Glycolipids, glycoproteins
Phosphorylation of proteins
Inositol lipids
Cytoskeleton
Genome
Nucleoli
Mitochondria

Surface charge
Surface enzymes
have clinical utility in early diagnosis and management of cancer patients. Earlier reports on serum levels of glycolytic enzymes like lactate dehydrogenase (LDH) and phosphohexose isomerase (PHI) have indicated their importance for early detection and management of cancer patients (Buchsbaum et al., 1991; Baumann et al., 1990; Patel et al., 1994; Singh et al., 1993).

Many of the tumour markers are the results of foetal or embryonic gene expressions. Undifferentiated cancer cells resemble foetal cells, both morphologically and biochemically. Therefore, cancer (onco) gene expression is frequently identical to the expression of the gene in embryonic tissues. This research area of oncodevelopmental biology focuses on the protein products, the RNA products, and regulation of expression of cancer genes. A variety of biomolecules, have expressed the potential for being effective tumour markers. The typical examples of these biomolecules are oncofoetal antigens, α-fetoprotein (AFP) (Abelev et al., 1963), carcinoembryonic antigen (CEA) (Gold and Freedman, 1965) and foetal isoenzyme such as placental like alkaline phosphatase (PLAP) (Fishman et al., 1968b; Koshida et al., 1991).

Taking all these aspects into consideration, the present work was undertaken to evaluate serum levels of following biochemical markers to assess their usefulness in early diagnosis and management of lung cancer patients and oral cavity cancer patients.
1. **GLYCOPEPTIDE CONSTITUENTS:**

   (I) **Sialic acid forms:**
   - Total sialic acid (TSA)
   - TSA/Total Protein (TP) ratio
   - Lipid bound sialic acid (LSA)
   - Free sialic acid (FSA)

   (II) **Other glycoprotein constituents:**
   - Fucose
   - Fucose/TP ratio
   - Hexosamines
     - Seromucoid fraction assayed in terms of its hexoses (galactose+mannose) and protein (mucoid proteins) contents.

2. **ENZYMES:**

   - Alkaline phosphatase (ALP)
   - Placental like alkaline phosphatase (PLAP)
   - Lactate Dehydrogenase (LDH)
   - Phosphohexose Isomerase (PHI)

   Combined use of different biomarkers is more significant than any single assay (Das et al., 1985; Ganz et al., 1987; Waalkes et al., 1983). As reported by Tautu et al. (1991) multiple marker analysis using combination of TSA and CEA appears to be more sensitive than CEA alone for detecting colorectal carcinoma. Similarly, Gail et al. (1986) suggested that combined use of TSA and CEA performed better than CEA alone in detection of advanced lung cancer. The combined use of fucose and sialic acid revealed high degree of marker positivity
in monitoring the patients with various malignancies (Turner et al., 1985). It has been suggested that, with combination assays in which one of the two markers is positive, the sensitivity and accuracy of the tests increased (Kurokawa et al., 1993). The combined evaluation of TSA, hexoses and CEA was found to be more useful in detecting malignant disease and recurrence during follow-up (Vehara et al., 1984). As mentioned by Schutter et al. (1992) LSA could be more useful for monitoring cancer patients under treatment, in combination with other established tumour markers like CEA or CA-125. Thus, previous reports by various authors have specified that combined use of the biomarkers enhanced the utility of each marker. Hence, an attempt was also made to determine whether multiple biochemical markers in different combinations are more useful for diagnosis and management of patients with cancer of the lung and oral cavity.

The present study included healthy individuals without any habit, healthy subjects with habit of either smoking or chewing of tobacco with clinically normal lung and oral cavity, patients with benign lung diseases as well as patients with oral premalignant conditions (pathological controls), lung cancer patients and patients with cancer of the oral cavity. The healthy individuals with or without any habit served as controls. The serum levels of the markers were analyzed in lung as well as oral cavity cancer patients before initiation of anticancer treatment and during/after treatment. The
sensitivity and specificity of the biomarkers were determined individually as well as in different combinations.

Considering all these vital aspects, the present study centered around the following major objectives.

(1) Evaluation of different sialic acid forms, other glycoprotein constituents and enzymes in non-smokers, normal smokers, patients with benign lung diseases (BLD) and untreated lung cancer patients.

(2) Determination of the values of sialic acids, other glycoprotein constituents and enzymes in non-chewers, normal chewers, patients with oral precancerous conditions (OPC) and patients with oral cavity cancer (OC).

(3) Comparison of the levels of biomarkers between benign/premalignant and malignant conditions.

(4) Correlation of the levels of different sialic acid forms, other glycoprotein constituents and enzymes with disease status in untreated lung and oral cavity cancer (OC) patients.

(5) Evaluation of individual sensitivity and specificity of the biomarkers in diagnosis of lung and oral cavity cancer.

(6) Determination of usefulness of the markers in diagnosis of
cancer patients with the help of Receiver Operating Characteristic (ROC) curve analysis.

(7) Evaluation of combined usefulness of the markers to derive more sensitive and specific combinations for the diagnosis of lung and oral cavity cancer.

(8) Observation of the alterations in levels of the biomarkers in sera of lung and oral cavity cancer patients after initiation of anticancer treatment.

(9) Evaluation of combined use of biomarkers in monitoring cancer patients during/after follow-up study.