5

DISCUSSION
The identification and application of quantifiable tumour markers as adjuncts to clinical care is a story of both, success and failures. The tumour markers are biological products secreted in circulatory system or expressed at surface of malignant cells. In this respect, the cell membrane is of paramount interest. It is the barrier between inner and outer phase of the cell which harbours the immunological and chemical density whose information must be transduced to the inert lipid bilayer (Nicolson, 1984). A variety of neoplastic changes are expressed at cell membrane and altered surface characteristics are essential for abnormal growth and behaviour of malignant cells (Nigam and Contero, 1973). Various cell membrane changes, e.g. modified glycoproteins and glycolipids, alterations in surface enzymes and other phenotypic changes have been associated with malignant transformation of a cell. Special attention has been given to define the biochemical changes at cell surface glycoproteins and glycolipids that take place during malignant transformation (Nicolson, 1984). Carbohydrate moieties of the cell surface glycoproteins may undergo additional changes including increase in sialic acid, fucose, hexosamine levels during neoplastic transformation (Yogeeswaran, 1983). The elevated concentrations of sialic acid, fucose, hexosamines and seromucoid fraction are often found in blood, through increased turnover, secretion and or shedding from malignant cells (Alhadeff, 1989).

Processes of neoplastic transformation often cause
structural modifications in the final product of the tumour tissue. Numerous modified biological substances have been secreted and detected in body fluids e.g. enzymes and isoenzymes tissue specific antigens, various metabolic products and oncofoetal antigens. These biological substances have been proposed as circulating tumour markers for various malignancies and are most feasible in clinical practice. The expression of oncofoetal antigens and the recognition of ectopic protein production have been the subject of intensive study during last few decades (Magdelenat, 1992). Among the increasing number of biochemical phenotypic markers from malignant cells, enzymes play an important role in cancer detection (Schwartz, 1989). Various attempts have been made to assess the clinical utility of enzymes for detection and management of malignant diseases (Schwartz, 1992). Alterations in serum enzymes are often reported in cancer patients. Among various serum enzymes, ALP, PLAP, LDH and PHI have received considerable clinical attention in confirming presence of cancer, in staging or in monitoring the cancer patients following anticancer treatment (Baumann et al., 1990; Berruti et al., 1993); Schwartz, 1992; Stigbrand et al., 1990).

Several reports have been published on increased levels of serum sialic acid, fucose, hexosamines, seromucoid fraction and enzymes like ALP, PLAP, LDH and PHI in patients with various malignancies. However, precise relevance of these biomarkers with disease status, pathology and follow-up in patients with
lung and oral cavity cancers have not been systematically studied. There is a very high incidence of cancer of the lung and oral cavity in India. The habit of tobacco chewing enhances the risk of oral precancerous lesions to significant degree. Similarly there is a wealth of evidence indicating damage done to lung tissue by cigarette smoking. The battery of biomarkers may be helpful in detecting malignancy at an early stage for cancer of the lung and oral cavity. These considerations provided a rationale for using serum sialic acids, other glycoprotein constituents and enzymes (ALP, PLAP, LDH and PHI) as possible tumour markers for detection and clinical management of lung and oral cavity cancers, the major tobacco associated malignant diseases.

The biomarker levels were compared between healthy individuals without any habit as well as individuals with habit of smoking or chewing tobacco to evaluate the possible effect of tobacco consumption. LSA, ALP and PLAP levels were significantly elevated in normal smokers compared to non-smokers. PLAP levels were also found to be significantly higher in normal chewers. Several workers have reported that increased PLAP levels are associated with smoking habit (Maslow et al., 1983; Muensch et al., 1986; Tonik et al., 1983). However, mechanisms behind elevations in LSA, PLAP or ALP in normal smokers or PLAP in normal chewers are not known.

Elevations in sialic acids and other glycoprotein constituents in the sera of cancer patients have been reported
by various investigators (Erbil et al., 1986; Gosh et al., 1988; Plucinsky et al., 1986 and Turner et al., 1985). The results of present study showed significant rise in serum sialic acid levels and other carbohydrate moieties in cancer patients compared to the controls. Serum sialic acid including TSA and LSA have previously been measured in lung cancer patients by Kakari et al. (1991). The authors have examined multiple markers including TSA, LSA, CEA, ferritin and neuron specific enolase (NSE) and found that TSA and LSA are the most useful markers for lung cancer diagnosis. Increased levels of TSA and LSA have been reported in patients with lung and other malignancies including oral cavity, ovary, colon, rectum as compared to normal subjects (Baxi et al., 1990; 1991; Dryfess et al., 1992; Gosh et al., 1991; Kakari et al., 1988; Patel et al., 1989; Silver et al., 1981). As reported by Verazin et al. (1990), TSA levels were significantly higher in only advanced colorectal cancer patients whereas, TSA/TP ratio showed significant elevations according to the different stages of colorectal cancer. They have also suggested that the TSA/TP ratio is a better marker than CEA for detection of colorectal cancer. TSA, LSA and TSA/TP levels have been found to be elevated in other malignant diseases as well (Horgan, 1982; Munjal et al., 1984; Plucinsky et al., 1986; Shamberger, 1984; Stefenelli, 1985). Thus, our results were in accordance with observations by various other workers.

It is apparent from comprehensive studies by various
investigations and the current study, that levels of these markers may provide clinical usefulness in detection of various malignancies. However, it should be noted that sialic acid and other glycoprotein constituents may become elevated in several other abnormal pathological conditions like acute inflammation, high fever, rheumatoid arthritis etc. (Macbeth and Bekesi, 1962; Voigtman et al., 1989). A high serum sialic acid concentration has been found in a patient with bacterial prostatis (Erbil et al., 1986). Serum glycoprotein constituents also increased in pulmonary tuberculosis (Singh et al., 1989). Therefore, an ideal tumour marker should have potentials to differentiate between benign/non-malignant and malignant diseases. Hence, the current investigation compared the levels of serum sialic acid forms and other glycoprotein constituents between controls and patients with benign/premalignant as well as between patients with malignant and non-malignant diseases. The results showed significant increase in the levels of sialic acid, fucose, fucose/TP, seromucoid fraction in patients with benign/premalignant diseases compared to the controls. Thus, observations suggest that biomarkers can serve as indicators of premalignant change in the patients with diseases of lung and oral cavity. Lung cancer patients had significant elevations in serum sialic acid and other glycoprotein constituents compared to patients with BLD. Likewise, levels of TSA/TP, LSA, fucose, fucoses/TP, hexosamines and seromucoid fraction were found to be significantly higher in OC patients compared to patients with
OPC. Thus, biomarkers evaluated were found to be useful in differentiating between benign/premalignant and malignant conditions.

Increased catalytic activity has been associated with malignancy without any obvious liver involvement. Elevated activity of ALP and PLAP was observed in lung and OC patients compared to controls in the present study. The reason for elevations in these two enzymes is largely unclear, however, it might be a reflection of systemic reaction of progressive disease (Harmenberg et al., 1989). Elevated ALP activity in serum might be increased in tumour involving bone or liver has been assessed, however, various investigators have reported utility of ALP for other malignancies also (Chodack et al., 1991; Das et al., 1985; Rao et al., 1978; Schwartz, 1989). Increased levels of PLAP has been observed in cancer of lung (Fishman et al., 1968b), ovary (Stigbrand et al., 1990), seminoma (Koshida et al., 1991) colorectal cancer (Harmenberg et al., 1989) and haematological malignancies (Patel et al., 1991; 1993a).

In the current investigation, serum levels of LDH and PHI were found to be significantly higher in lung cancer patients and OC patients compared to the controls. Elevated activity of serum LDH have been found in patients with colorectal cancer (Kemeny and Braun, 1983), lymphoma (Kornberg and Polliac, 1980), neuroblastoma (Quinn and Altman, 1980), testicular cancer (Lippert and Javadpour, 1981), leukemia (Patel et al., 1994) and
lung carcinoma (Rotenberg et al., 1988). Multiple factors are responsible for the elevations in LDH activity in malignant tumours among the few studies assessing the usefulness of LDH in monitoring the progress of patients with lung cancer, Gill et al. (1981) found positive correlation between progression of malignant disease and LDH activity. Cohen et al. (1981) reported a significant correlation between higher LDH activity and a worse prognosis in small-cell lung cancer. A significant correlation between disease status and serum LDH activity in lung cancer patients was observed by Aroney et al. (1984). Alterations in PHI levels have received considerable attention during recent years. Several authors have reported increased PHI levels in various malignancies (Baumann et al., 1988; 1990; Das et al., 1985; Hennipman et al., 1988; Ho et al., 1982; Santabarbara et al., 1988). The value of PHI as a tumour marker is of particular interest since Chaput et al. (1988) and Faik et al. (1988) reported that PHI functions as a tropic factor related to proliferative activity in malignant tumours. The enzyme activities of ALP, PLAP, LDH and PHI were found to be evaluated in lung cancer and OC patients as well as in patients with BLD and OPC. Significant elevations were observed in the levels of ALP, PLAP, LDH and PHI in lung cancer patients compared to patients with BLD. Whereas, in OC patients only PLAP levels were significantly raised compared to patients with OPC. Significant elevations were observed in the levels of ALP, PLAP, LDH and PHI in lung cancer patients compared to patients with
OPC. The results suggest that serum ALP, PLAP, LDH and PHI values were useful for differentiation between lung cancer and patients with BLD. On the other hand among the enzymes studied, only PLAP levels could successfully differentiate between OC patients and patients with OPC.

Serum sialic acid and other glycoprotein constituents as well as ALP levels were found to be significantly increased with progression of oral cavity cancer (Figure-25, table-33, table-34). The present study included a less number of OC patients with stage-I (n=3). Therefore, the statistical comparison was studied only between stage-II, stage-III and stage-IV OC patients. Stage-III OC patients showed significant rise in LSA and hexose levels than stage-II OC patients. TSA, TSA/TP, LSA, fucose, fucose/TP, hexosamines, mucoid patients and ALP levels were significantly elevated in stage-IV OC patients compared to stage-III OC patients. Thus, when OC patients were classified according to the stage of the disease, the highest levels of serum sialic acid, other glycoprotein constituents and ALP were found among patients with cancer in advanced stage. Various investigators have reported significant correlation of alterations in the levels of sialic acid and other glycoprotein constituents with stage of the disease and tumour burden in patients with cancer of the ovary, breast and oral cavity etc. (Baxi et al., 1991; Dryfess et al., 1992; Gosh et al., 1991; Hogan-Ryan et al., 1980; Plucinsky et al., 1986; Salvango et al., 1985; Turner et al., 1985; Waalkes et al., 1983). ALP has
been found to be useful to confirm the presence of cancer, in staging and to monitor the presence of metastases (Schwartz, 1989). Present findings are in agreement with results reported by Berruti et al. (1993) and Schwartz (1976).

When lung cancer patients were grouped according to spread of the disease, serum sialic acids, other glycoprotein constituents and enzymes were increased in higher number of patients with extensive disease than limited disease. Furthermore, OC patients with metastases showed significant rise in the levels of TSA, TSA/TP, LSA, fucose, hexosamines and mucoid protein compared to patients with primary cancer. It has been reported that total cell sialic acid, total ganglioside content and sialic acid bound to glycoprotein oligosaccharides are increased in various types of transformed cells and may reflect a general increase in cell surface negative charge. More recent studies suggest that the cell surface sialic acid and degree of sialylation correlate with metastatic potential of different types of cancers. Elevated cell surface sialoglycoconjugates seen in malignant may influence several properties including arrest of blood born tumour cells, adhesion to blood vessel endothelium, implantation, survival and growth at secondary sites which facilitate tumour cell metastases (Nicolson, 1984; Yogeeswaran, 1983). Waalkes et al. (1978) reported that sialic acid, fucose bound glycoconjugates and the levels of CEA were the most useful markers following the course of human metastatic breast carcinoma. The levels of serum
sialic acid, serum protein bound neutral hexoses and fucose have been shown to be frequently higher in majority of patients with metastatic cancer (Apffel and Peters, 1979; Dnistrain et al., 1982; Erbil et al., 1986; Katopodis et al., 1982; Plucinsky et al., 1986; Waalkes et al., 1983). Elevated activity of PLAP in advanced stage of colorectal cancer was reported by Harmenberg et al. (1989). West et al. (1962) reported raised levels of PHI in patients with lung cancer, with lower values in patients without distant metastases and highest values in those with hepatic metastases. Thus, significant correlation between PLAP and PHI levels and metastases have been observed in malignancy.

An ideal tumour marker should be sensitive not only to differentiate between controls and cancer patients but it should be specific to differentiate between patients with non-malignant and malignant diseases also. Considering this, specificity and sensitivity of the markers were studied. TSA was found to be the most sensitive (98.6%), while the sensitivity of LSA was maximum (75.3%) for diagnosis of oral cavity cancer. Sialic acid assays have been recommended to be highly sensitive marker for breast cancer (Dwivedi et al., 1990), lung cancer (Munjal et al., 1984), colorectal cancer (Dnistrain et al., 1981) and intracranial tumours (Marth et al., 1988). LSA and TSA was found to be most sensitive marker for diagnosis of lung carcinoma (Kakari et al., 1991). As reported by Klapan et al. (1993) LSA was highly sensitive (94.4%) for head and neck cancer diagnosis. The authors analyzed the validity (sensitivity as
well as specificity) of LSA, prostaglandin E and histamine as diagnostic markers and found that LSA was the most sensitive and specific marker for detection of head and neck cancer. Present findings on preoperative LSA levels in cancer of the oral cavity are in accordance with those of Katopodis et al. (1982) who have demonstrated high sensitivity (77%-97%) of the LSA test in higher number of patients with breast, colon and lung carcinoma. In patients with laryngeal cancer also PLAP was found to be most sensitive marker (Mavio et al., 1991). Among the glycoprotein constituents examined, the hexosamine levels were found to be the most specific marker for lung (93.4%) and oral cavity (94.3%) cancers. However the sensitivity of hexosamine for diagnosis of lung and oral cavity malignancies is very low which limits the utility of hexosamines as marker for the diagnosis of cancer of the lung and oral cavity.

When diagnostic utility of ALP, PLAP, LDH and PHI was determined for lung and oral cavity cancers, PHI was found to be the most sensitive (78.6%) and ALP was found to be the most specific (90.2%) marker for diagnosis of lung cancer. Similarly, for diagnosis of oral cavity cancer, the sensitivity of PLAP was maximum (79.2%) and LDH was found to be the most specific (92.4%) marker. PHI was found to be most sensitive (70.0%) marker in gastrointestinal malignancy also (Baumann, et al., 1990). Schwartz et al. (1985) found high sensitivity (97.0%) and good specificity (94.0%) of PHI for diagnosis of lung cancer and suggested that PHI is an useful marker in early
detection of lung cancer. In contrast D’Eril et al. (1986) reported that PHI had only fair sensitivity (73.0%) and a very low specificity (15.0%) for diagnosis of lung cancer. In the present study the sensitivity and specificity of PHI were found to be within the realm of clinical usefulness and compared well with the generally reported values.

PLAP showed maximum sensitivity (79.2%) for diagnosis of oral cavity cancer. To the best of our knowledge very few reports have been published concerning diagnostic utility of PLAP in head and neck cancer. (Deyasi et al., 1976; Muensch et al., 1986). PLAP exhibited some promise as tumour marker for breast and genitourinary cancer (Cadeau et al., 1974), for ovarian cancer (Chen and Hsu, 1985) and for testicular germ cell tumours (Koshida et al., 1991).

The results of the present study suggested that though hexosamines, ALP and LDH were found to be highly specific markers they lacked sensitivity. Looking to the results, it is necessary to consider both sensitivity and specificity to find out the best useful markers. The use of ROC curve analysis facilitates the choice of a cut off value and allows simultaneous evaluation of specificity and sensitivity of different diagnostic markers (Feinstein, 1985). ROC curve analysis have gained increasing popularity in recent years (Zweig and Campbell, 1993). It has several advantages over other statistical evaluations including student’s ‘t’ test for diagnosis of malignancy. ROC curve is a graphical
representation which can provide a comparative assessment of the markers.

During recent years clinical assessment of serum sialic acid has proved to be promising markers for cancer of the lung and oral cavity (Baxi et al., 1991; Kakari et al., 1991; Patel et al., 1989; Xing et al., 1991). However, evaluation of these markers is often difficult because of variance and heterogeneity of reference population and perhaps this is one of the reasons why role of these markers in lung and oral cavity malignancies is not yet well established. The use of ROC curve analysis is the best way to present data relating to the serum levels of marker to the presence or absence of a tumour. In the current study, serum sialic acids, other glycoprotein constituents and enzymes were analyzed with the help of ROC curves to assess their efficacy as diagnostic markers for lung and oral cavity cancers. Among all the parameters, the ROC curves for TSA, TSA/TP, LSA, hexoses, mucoid proteins and PHI exhibited higher locations on the graph (i.e. higher diagnostic accuracy) compared to other biomarkers for diagnosis of lung cancer. Utilizing ROC curves, sensitivities of all the markers were determined at specificity levels between 60-90%. TSA, TSA/TP, LSA, hexoses and PHI revealed considerably higher sensitivities at different specificity levels. Likewise, in detecting oral cavity malignancy, TSA, TSA/TP, LSA, hexoses, mucoid proteins and PLAP were found to be more useful markers as analyzed by ROC curves. Sensitivities of these useful markers
were strikingly higher than remaining markers at various specificity levels. ROC curve analysis revealed, FSA, fucose, fucose/TP, hexosamines, ALP and LDH as less useful markers. Sensitivities at specificity levels between 60-90% also suggested the inadequacy of these markers as diagnostic indicators for lung and oral cavity malignancies.

Various authors have reported that multiple markers, if applied in different combinations, are more useful rather than any single assay (Gail et al., 1988; Patel et al., 1991; Tautu et al., 1988; Uehara et al., 1984; Walker and Gray, 1983). Taking this in to consideration, combined analysis of most useful biomarkers was performed. Combined use of serum sialic acids (TSA or LSA) either with hexoses or with PHI revealed maximum sensitivity (99.3%) for diagnosis of lung cancer. The validity (specificity and sensitivity) of diagnostic assays including sialic acid, hexoses and PHI was comparatively lower, when these parameters were used alone. Combination of TSA/TP either with seromucoid fraction or with PLAP was found to be most specific (95.2%) for diagnosis of oral cavity cancer. The combination of mucoid proteins with PLAP revealed maximum sensitivity (97.0%) for detection of oral cavity cancer. Numerous other workers, strongly advocated combined usefulness of multiple biomarker assays, for cancer detection (Gail et al., 1987; Ho et al., 1982; Santabarbara et al., 1988; Tautu et al., 1991; Turner et al., 1985). The present findings also support the contention that analysis of tumour markers if used in
various combinations are more meaningful.

The use of tumour markers depends not only upon their sensitivity and specificity but also upon their ability to influence management decisions. Useful markers are those which specifically identify the viability of a malignant process, even at a preclinical stage. Such markers are particularly important in lung cancer detection which is difficult to evaluate because of its insidious nature. Furthermore, once the disease is diagnosed and anticancer treatment is administered, it is necessary to have evaluation of parameters which correlate with the disease status and the treatment response. Incidence and mortality data for lung cancer showed a persistently rapid rise of the disease (Szabo et al., 1993). Simultaneously there is a lack of objective parameters which can be helpful in treatment monitoring of OC patients. Therefore, in the present investigation, the multiple biomarker were used to predict the response to the anticancer therapy.

The current study also evaluated serum sialic acids, other glycoprotein constituents and enzyme levels to find out their clinical value in treatment monitoring of lung cancer patients and OC patients. Pretreatment levels of all the biomarkers were significantly higher in lung cancer patients and OC patients compared to the controls. To assess the therapeutic response to anticancer treatment, comparisons of the biomarker levels were made between their pretreatment values and follow-up levels in responders as well as in non-responders. The biomarker was
considered to be useful for evaluation of efficacy of treatment when the marker levels significantly decreased in responders as well as same biomarker significantly increased in non-responders compared to their pretreatment levels. In lung cancer patients serum levels of TSA, TSA/TP, LSA, seromucoid fraction and PHI were significantly correlated with disease response in patients receiving anticancer treatment. As reported by Kokoglu et al. (1989) serum sialic acid levels are valuable tool in detecting risk of recurrence and evaluating therapeutic response in patients with thyroid cancer. In another study, Shamberger (1984) found decrease in sialic acid levels, following the removal of tumour. Stringou et al. (1992) have measured serial estimations of TSA, LSA and CEA in four large groups of cancer patients including bladder, lung, uterus and breast cancers. Their results proved sialic acid as an important biochemical marker for assessing response to treatment of cancer. Sensitivity of sialic acid to judge response to the treatment was 15% higher than that of CEA. Other investigators have reported that sialic acid may accurately monitor treated patients for early detection of recurrence (Patel et al., 1990b; Silver et al., 1989; Toner et al., 1990). Evans et al. (1974) have reported that the patients who were not responding satisfactorily to therapy showed elevations in both hexoses and fucose levels, while patients without demonstrable tumour burden tended to normal levels of both the parameters. Serum sialic acid (TSA, TSA/TP and LSA) and seromucoid fraction were
significantly correlated with disease status during follow-up in OC patients. The levels of markers significantly declined with remission of the disease. Whereas, non-responders showed significant elevations in serum sialic acid and seromucoid fraction as compared to their pretreatment levels. Xing et al. (1991) suggested that serum sialic acid may be useful in monitoring the patients with oral cavity cancer. The results of present study found that serum sialic acid, and seromucoid fraction can be used as a marker of tumour activity during follow-up of the patients under treatment. In addition, elevations in biomarker levels in non-responders or consistently higher levels of the markers may indicate a poor prognosis of the patient.

PHI activity correlated well with tumour burden as well as therapeutic response to the treatment in lung cancer patients (figure-16, figure-17, table 21). Other workers have also reported that PHI have potential use in treatment monitoring of cancer patients (Das et al., 1985; Rao et al., 1976). As reported by Santabarbara et al. (1988) serial determination of PHI was useful for follow-up of the patients with lung carcinoma. Thus, PHI can be used as an indicator to predict cancer patients at high risk for recurrence. However, none of the enzymes showed any significant correlation with therapeutic response in OC patients.

It has been suggested that combined utility of biomarkers may be more helpful in early detection of recurrence (Uehara et
al., 1984). Combined use of TSA and LSA as well as mucoid proteins and PHI were found to be the most beneficial in evaluating therapeutic response in lung cancer patients receiving anticancer treatment. In OC patients combinations of TSA/TP either with hexoses or with PLAP were more useful for monitoring the treatment. In non-responders, all possible combinations of more useful markers were found to be higher than the cut off limit in all the patients. Thus, the results strongly suggest that combined utility of more useful biomarkers is more practical to assess disease activity during treatment in lung cancer and OC patients.

In a nut shell, the measurement of serum sialic acid, other glycoprotein constituents as well as enzymes have clinical usefulness in diagnosis of lung and oral cavity cancers. Simultaneous quantification of more useful markers significantly improve their sensitivity and specificity. The biomarkers evaluated hold promise as supplement marker for staging and in monitoring the invasiveness of the disease. The results suggest that these biomarkers have clinical utility in monitoring the disease activity during treatment and for evaluating the effectiveness of various therapeutic approaches. Thus serum sialic acid, other glycoprotein constituents and enzyme levels could be used to verify clinical staging, to identify those patients who should be considered at high risk of recurrence of the disease and to alert the clinician to the possibility of unrecognized occult disease.