AN OVERVIEW OF BIOCHEMICAL MARKERS IN MALIGNANCY
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2.1 Glycoproteins

2.2 Enzymes
Scientists and physicians have been searching for specific, reliable and easily identifiable markers for cancer. Uptill now no such single marker has been found. However, various biological substances are found to be elevated in cancer tissues and body fluids of cancer patients, which can be used as markers for different malignancies. These include (i) antigens, (ii) ectopically produced hormones, (iii) enzymes and isoenzymes, (iv) proteins, (v) products of cellular metabolism and (vi) antigens defined by monoclonal antibodies (Magdelenat, 1992; Ozer et al., 1987). Qualitative and quantitative assessment of these markers are currently utilized:

(a) to identify the possibility of the presence of neoplasm,
(b) to detect carcinomatous formation in its early stage,
(c) to establish prognosis,
(d) to monitor the response to anticancer treatment,
(e) to evaluate disease status of the patients in clinical remission or recurrent progress and
(f) to avoid a second look surgery in majority of the cases.

The first such marker was identified over 140 years ago, when H. Bence Jones described that the Bence Jone's protein, was elevated in the plasma of patients with multiple myeloma. The second era of tumour markers from 1928 to 1963, included the discovery and application of hormones, enzymes and isozymes for cancer diagnosis. However, the era of tumour markers as more generally applicable tools began with the discovery of α-fetoprotein (AFP) by Abelev in 1963 and carcinoembryonic
antigen (CEA) by Gold and Freedman in 1965. This approach raised a great hope of finding out other specific 'tumour markers'. In the past decade, alterations in glycolipids and glycoproteins as well as ectopic production of proteins like foetal antigens by tumour cells and several glycolytic enzymes were found to be the most clinically useful tumour-associated markers (Ozer et al., 1987; Schwartz, 1992; Smets and Van Beek, 1984).

2.1 **Glycoproteins:**

Glycoproteins have been recognized as an important group of compounds found in all forms of life. The abundant amount of glycoproteins are found in cell surface, internal membranes and in extracellular matrix such as basement membrane (Kornfeld and Kornfeld, 1980). Glycoproteins are usually defined as a heterogeneous group of protein-carbohydrate complexes in which oligo or polysaccharides are joined by covalent linkage to specific aminoacids. The appearance of these glycoconjugates in sera of tumour bearing animals and humans is bound to have diagnostic and prognostic value and may also be important determinants in pathophysiology of cancer (Smets and Van Beek, 1984). Alterations in cell surface membrane bound glycoproteins have been demonstrated in transformed cells and tumours (Klapan et al., 1993; Magdelenat, 1992; Ogoshi et al., 1992; Polivkova et al., 1988; Stringou et al., 1992). Several attempts have been made to determine whether cancer-related alterations in surface carbohydrates can also be detected in tumours in vivo or
in freshly explanted tumours (Smets and Van Beek, 1984). In view of the correlation between glycosylation differences and the tumourogenic potentials, the possible clinical application of these biochemical changes as a diagnostic tool should be considered (Yogeeswaran, 1983). The cell surface glycoproteins may undergo additional changes in carbohydrate components. The major carbohydrate components of glycoproteins are D-galactose, D-mannose, the N-acetyl derivatives of D-glucosamine and D-galactosamine, L-fucose and sialic acid (Nigam and Contero, 1973). These carbohydrate moieties of glycoproteins may influence growth and cell to cell interaction and thus may be important in development of malignancy (Hakomori, 1981; Hakomori, 1984). They affect adherence, cohesion and antigenic expression by virtue of outstanding capacity for columbic repulsion and binding of water on the tumour cell surface. Changes in the serum sialoglycoproteins that profile malignancies are shared by other disease states, but correlation of malignant cell with increased or abnormal sialoglycoproteins appear to be different (Alhadeff and Holzinger, 1982; Alhadeff, 1989; Bolmer and Davidson, 1981; Feizi and Childs, 1987; Schauer, 1988).

The process of shedding and increased turnover of surface components in normal cells is natural physiological phenomenon accompanying growth and cell proliferation (Black, 1980). Cancer cells also continuously shed their surface components during malignant process. The phenomenon of shedding may be
mediated by an increase in cell surface glycoproteins (Yogeeswaran, 1983). Glycosylation changes in tumour cells are generalized, however it is unknown whether the increase in circulating sialoglycoproteins in malignant diseases are derived from cell lysis or extra sialylated molecules of tumours. Certain findings regarding glycoproteins in human cancer are conflicting (Cohn et al., 1986; Maity et al., 1983). Inspite of these contradictory reports, differences in the chemical make up of the cell surface of normal and tumour cells have revealed that there are consistent patterns of alterations with regard to glycoproteins and glycolipids. Quantitative measurement of circulating glycoconjugates might be helpful in detecting progression of malignant disease in large population of patients (Kakari et al., 1991; O’Kennedy et al., 1991; Turner et al., 1985). The information regarding alterations in sialoglycoproteins may be useful in differentiation of patients with non-malignant and malignant diseases and in monitoring the patients during treatment (Marth et al., 1988; Ogoshi et al., 1992; Schutter et al., 1992; Shamberger, 1984; Stefanelli et al., 1985; Verazin et al., 1990).

It is clearly evident from the available literature that glycoproteins have a vital role in number of cell functions. These glycoproteins are hormones, enzymes, receptors, antigens, cell surface mucins, carbohydrate or blood group related antigens, which seem to be abnormally expressed in malignant cells. Human chorionic gonadotropin, thyroglobulins
(receptors), glucoamylase, betahexosaminidase, gamma glutamyl transpeptidase (enzymes) are glycoprotein in nature (Kobata et al., 1984). Another examples of certain glycoproteins are tumour specific antigens such as pancreatic cancer associated antigens, ovarian cancer antigen, prostate specific antigen (Ozer et al., 1987). Glycoprotein functions as receptors (e.g. acetylcholine receptor, low density lipoprotein receptors) (Kobata, 1984). P-glycoprotein has been described as drug resistant glycoprotein, which is present in number of cells including tumour cells. P-glycoprotein could provide a diagnostic basis for planning the treatment of cancer patients (Bell et al., 1987; Kartner et al., 1983). Alterations in cell surface membrane bound glycoproteins can be classified into three types (i) a loss of high molecular weight glycoprotein (e.g. fibronectin), (ii) an increase in certain cell surface glycoproteins in lymphoproliferative diseases and (iii) an increase in novel glycoprotein such as epiglycanin (Yogeeswaran, 1983). Fibronectin is an adhesive glycoprotein that has been shown to decrease in extracellular matrix or transformed cells (Yamada et al., 1985). Laminin is also another example of glycoprotein, which is also an adhesive glycoprotein and it is believed to be involved in the metastatic spread of cancer (McCarthy et al., 1985). Epiglycanin is large, mucus type, intrinsic membrane glycoprotein isolated from mammary adenocarcinomas (Codington et al., 1975). Moreover, increasing evidence support that the carbohydrate moieties of cell surface
glycoproteins may undergo additional changes during malignant transformation e.g. sialic acid, hexoses, fucose, hexosamines (Feizi and Childs, 1987; Schauer, 1988). Several glycoproteins appear in embryonic or foetal tissue, same glycoproteins may reappear in tumour tissue. These glycoproteins are well characterized oncofoetal antigens, such as CEA, AFP and placental like alkaline phosphatase (PLAP) (Hirai, 1979) which have been used extensively as biochemical markers for the diagnosis, staging and treatment monitoring of various malignancies (Cohn et al., 1986; Frische et al., 1985; Gustafsson et al., 1988; Patel et al., 1993a; Vergote et al., 1992; Yamamoto et al., 1993).

The glycoprotein constituents including (i) sialic acid forms, (ii) fucose, (iii) hexosamines and (iv) seromucoid fraction were selected as biochemical markers in the present study to determine their efficacy in diagnosis and treatment monitoring of lung and oral cavity malignancies.

SIALIC ACIDS:

Sialic acid is the generic term given to a family of acetylated derivatives of neuraminic acid. As suggested by Schauer (1985), sialic acids participates in major biological functions. For example, sialic acids play a role in cell biology by their negative charge, and associate in the transport and binding of positively charged molecules. Further, it influence the confirmational states of glycoproteins, by acting as receptors for microorganisms, toxins and hormones as well as
by masking receptors and other immunological, recognition sites of molecules and cells. The later function attracts more interest as it seems to be of outstanding importance (Schauer, 1988). Sialic acids occur widely in nature as in free form or bound to oligosaccharide chains of membrane bound glycoproteins and glycolipids. They usually bound to sugar group such as fucose, galactose or N-acetyl glucosamine which themselves are attached to glycerol derivatives by glycosidic bonds. The sialic acid moiety generally occupy a terminal position of, oligosaccharide side chains, however, internal sialic acids have also been found within these chains in both glycoproteins and glycolipids such as ganglio sides (Crook, 1993). Sialic acid as a part of membrane glycoproteins and glycolipids, is assumed to pass into circulation by shedding or cell lysis. The total serum concentration of sialic acid depends not only on the concentration of serum glycoproteins or glycolipids, but also the degree of sialylation of these substances (Yogeeswaran, 1983). A substantial part of the serum sialic acid is bound to acute phase proteins such as \( \alpha_1 \)-acid glycoprotein, \( \alpha_1 \)-protease inhibitor and hepatoglobin and it therefore reflects cytokine-induced changes in biosynthesis and posttranslational glycosylation process of the acute-phase glycoproteins in the liver (Pos et al., 1990; Van Dij et al., 1991). Sialic acid influences physicochemical properties of glycoproteins, participates in homeostatic functions and affects the specificity of blood group antigens and invasiveness of tumour
cells (Oztokatli et al., 1991). Changes in sialic acid contents have been observed in inbred mice bearing tumours induced by benzopyrene. Significant increase in the levels of lipid bound sialic acid (LSA) have been observed in the plasma of suspected mice even before the tumour was not macroscopically ascertained (Polivková et al., 1988).

Since sialic acids are major constituents of glycoproteins and glycolipids, several investigators have studied their levels in the serum/plasma of patients with malignant diseases. Most of these studies have been concerned with total sialic acid (TSA) and/or LSA levels. The former value includes glycoprotein and glycolipid bound sialic acid as well as small amount of free sialic acid (FSA), whereas, the later value include only glycolipid bound sialic acid (Plucinsky et al., 1986). Erbil et al. (1985) have reported correlation of TSA and LSA with the stage and grade in patients with advanced urologic cancer and have also correlated levels of both TSA and LSA with tumour activity during treatment. Verazin et al. (1990) have studied TSA and TSA normalized to total protein (TSA/TP) in patients with colorectal cancer and have found that TSA/TP ratio may be helpful in early detection of colorectal cancer. Kakari et al. (1991) have studied multiple markers in lung carcinoma and have concluded that TSA and LSA were the best of tested markers for lung cancer diagnosis. Several studies have correlated TSA and LSA levels with tumour burden (Dryfess et al., 1992; Klapan et al., 1993; Polivková et al., 1992; Salvagno et al., 1985; Tautu
et al., 1991; Xing et al., 1991). TSA and LSA have been found to be useful in assessing the extent of malignant diseases and the response to therapy. In addition, they may accurately monitor treated patients for early detection of recurrence (Ogoshi et al., 1992; Patel et al., 1990b; Silver et al., 1989; Toner et al., 1990; Verazin et al., 1990). Serum TSA levels have been found to be useful in differentiating between patients with benign and malignant intracranial tumours (Marth et al., 1988). Several other workers have reported significance of TSA and LSA levels for diagnosis and monitoring of leukemia patients (Asami et al., 1987; Katopodis et al., 1982; Patel et al., 1991). Ozotokatli et al. (1991) have reported significance of LSA in bladder tumours. The authors found that variations in LSA values may indeed be helpful in monitoring the efficacy of therapy and in determining relapse during follow ups. Significantly increased levels have been found to be useful in tumour staging among breast cancer patients (Dwivedi et al., 1990; Patel et al., 1990b; Riley et al., 1990; Shanmugam and Nagarajan, 1987). LSA measurement appeared to be of limited value in the assessment of breast cancer but serial measurement might be useful in assessing disease progression and in identifying patients who were resistant to therapy (Dnistrain et al., 1982). Asami et al. (1987) have observed elevations in sialic acid levels in CSF of childhood leukemia patients with involvement of central nervous system compared to patients without central nervous system involvement and other
neurological diseases. A significant increase in sialic acid concentration have been found in patients with malignant central nervous system tumour (Gatchev et al., 1993). Kakari et al. (1984) have suggested that measurement of levels of TSA and LSA in CSF may be useful for staging the patients with pituitary adenoma and monitoring the patients for effectiveness of treatment. The metastases of malignant cells from the primary tumour to distant sites is probably the important event in cancer. Some studies have shown that cells of a high metastasizing line had 80% more neuraminidase susceptible sialic acid on its cell surface than cells of a low metastasizing line (Yogeesswaran et al., 1978). Yogeesswaran, (1983) had shown positive correlation between content of TSA of murine tumour cells grown in culture and its ability to metastasize spontaneously from subcutaneous sites. Serum sialic acid forms are clinically useful for detection of various malignancies and management of cancer patients receiving anticancer treatment (Dryfess et al., 1992; Ginsbourg et al., 1986; Klapan et al., 1993; Kokoglu et al., 1989; Patel et al., 1993b). However, the specificity of sialic acid as a marker in malignancies has been a matter of diversity, because increase in sialic acid was observed in several non-malignant conditions also (Cohn et al., 1986; Kakari et al., 1991; Maity et al., 1983; Raynes, 1983).

OTHER GLYCOPROTEIN CONSTITUENTS:

The carbohydrate moieties at the non-reducing ends of the glycoproteins are essential for their functions. Like sialic
acids other glycoprotein constituents including fucose, hexosamines and seromucoid fraction (hexoses and mucoid proteins) have been found to be elevated in patients with neoplastic diseases (Dang et al., 1985; Gosh et al., 1991; Snyder and Ashwell, 1971; Waalkes et al., 1978).

L-fucose, a methyl pentose has been found at the terminal non-reducing end of oligosaccharide chain of glycoproteins. It is most commonly associated with serum glycoconjugates (Neuberger et al., 1966). The tumour itself may also contribute to circulating fucose concentrations either by spontaneous release of glycoproteins with increasing burden of the tumour or as a result of cell damage by host attack or treatment (Turner et al., 1985). Numerous studies have shown that malignant changes may be accompanied by increased expression of membrane associated fucose containing macromolecules (Glick, 1978, Gosh et al., 1988; Hakomori, 1985; Kim et al., 1982; Miyauchi et al., 1982; Turner et al., 1980; Vischer et al., 1978). Different theories have been advanced to explain elevations in fucose levels. The possibilities to account for the increase in serum fucose content may be due to depolymerization of the ground substance of the connective tissue or surgical trauma, severe inflammation and tissue necrosis or may be due to hyperplasia of the cells (Tatsumura et al., 1977). It is stressed that the precise mechanism of raised fucose levels is still obscured. The raised fucose value in malignant disease reflected either a local or systemic tissue response to the tumour or arises from
the tumour itself. Tumours might also contribute indirectly to serum fucose by promoting increased fucosylation of existing glycoproteins (Turner et al., 1985). Positive correlation has been found between the stage of carcinoma and the magnitude of elevation of the serum fucose levels (Gosh et al., 1988). In small cell carcinoma of lung, serum fucose levels were found to be elevated which correlated directly with stage and extent of the disease (Waalkes et al., 1983). The protein bound fucose has been reported to be elevated in various malignant diseases and has been found useful parameter for diagnosis and prognosis of the disease (Dutta et al., 1976; Dang et al., 1985; Evans et al., 1974; Mishra et al., 1988; Turner et al., 1985). Sakai et al. (1990) have assayed free urinary L-fucose from patients with cancer and other non-malignant diseases and from healthy subjects to establish whether L-fucose excretion rate was different when a disease was present. Their results showed that L-fucose values were significantly higher in cancer patients than that in patients with benign diseases or healthy subjects. Barlow and Dillard (1972) reported high levels of serum fucose in gynecological cancers. Dutta et al. (1976) have found highest mean serum fucose levels in carcinoma of the cervix compared to other malignant tumours. The patients with breast carcinoma showed increase in serum fucose levels with the progression of malignant disease (Mishra et al., 1988). Elevated serum fucose levels have been observed in certain non-malignant diseases like, sub-acute bacterial endocardities,
parenchymatous liver diseases, tubercular meningitis, rickets, cirrhosis and gastric ulcers, trauma, severe inflammation and tissue necrosis (Turner et al., 1985; Tatsumura et al., 1977). Several other reports indicated that determination of serum fucose to protein ratio might be of distinct value as an adjunctive procedure in the diagnosis of malignant diseases (Dutta et al., 1976; Waalkes et al., 1978).

Sen et al. (1984) have measured serum fucose levels in mice bearing transplanted tumours, in order to investigate the relationship between these levels and the progress of transplanted tumour system. They observed correlation of serum fucose values with progression or regression of tumour growth rates. Evans et al. (1974) have emphasized utility of L-fucose for estimation of malignant tumour extension and for evaluation of efficacy of therapy in different types of malignant diseases.

Serum hexosamine (glucosamine and galactosamine) levels have been shown to be related to tumour burden in a group of patients with solid tumours (Bradley et al., 1977). Alpha-1 acid glycoprotein contains 12.4% hexosamine with glucosamine and galactosamine in proportion about 3:1. Inhibition of tumour cell specific agglutinin by hexosamines and their acetyl derivatives suggested that some changes in the glycoproteins, or their carbohydrate moieties, present on the surface membrane, might have taken place following malignant transformation (Kuroda, 1974). Serum hexosamine values were found to correlate with cancer diagnosis and management of cancer patients (Bradley...
Seromucoid, a carbohydrate rich fraction can be defined as the fraction of serum proteins, which is not precipitated with 1.8M perchloric acid but gets precipitated with 5% phosphotungustic acid (Thaw and Albutt, 1980; Varley et al., 1984). It has been studied as an indicator of tumour presence (Harshman et al., 1974; Winzlar, 1955). The seromucoid content of serum has been measured in terms of its protein contents (mucoid proteins), hexose content, tyrosine content or hexosamine content (Winzlar, 1955). Changes in glycoproteins can also be measured by determining seromucoid bound carbohydrates. MacBeth and Bekesi (1962) have determined the plasma protein bound hexoses (galactose and mannose) in the natural glycoprotein fraction and found that the levels were elevated above normal value in cancer patients. Further they suggested that the elevations in serum hexose levels were indicative of dissemination of malignant disease and it was also possible to follow response to treatment, by serial determination of plasma protein bound hexoses. The occurrence of elevated levels for serum protein-bound carbohydrates in patients with neoplastic diseases has been known and investigated over a period of years (Apffel and Peters, 1979; Evans et al., 1974; Snyder et al., 1971). Serum hexoses including mannose and galactose were elevated in blood of patients with small cell lung cancer which correlated with stage of the disease (Waalkes et al., 1983). Serum protein bound
hexose levels alone reported to be a far more reliable marker for the presence of malignancy than a combination of the acute phase reactant proteins (Walker et al., 1983). Furthermore, the authors reported that serum hexoses may have a clinical role in monitoring patients with cancer of the colon and rectum at high risk for developing recurrence. However, these protein bound hexoses have been found to increase in patients with non-malignant diseases also (Winzlar, 1955). The lack of specificity of these protein bound carbohydrates for cancer screening procedures does not preclude their utility in the sera of management of patients with established diagnosis of malignant disease. Evans et al. (1974) have reported usefulness of serum protein bound neutral hexoses for estimation of malignant tumour extension and evaluation of efficacy of therapy. Serum levels of seromucoid fraction may be of adjunctive value in assessing tumour burden and immune reactivity in cancer patients (Bradley et al., 1977).

Serum protein bound carbohydrates including sialic acid, fucose, hexosamines and seromucoid fraction have been examined by numerous investigators as possible sources for differentiating malignant disease from non-malignant diseases. As a result, a large volume of literature has accumulated over the last two decades, centered largely around the illustration of altered glycoproteins. Therefore, it is worthwhile to investigate usefulness of carbohydrate moieties of glycoproteins like sialic acid, fucose, hexosamines and seromucoid fraction
with respect to diagnosis, classification, staging, prognostication and treatment monitoring of patients with lung and oral cavity cancer.

2.2 **Enzymes:**

Enzymes, organic catalysts that are responsible for most chemical reactions in the body, are found in all tissues. Several enzymes have been identified in serum/plasma. The use of enzymes in serum have been studied as possible early indicators of neoplasia and as an aid in determining the progression or regression of the disease. There are number of possible mechanisms for the appearance of abnormal activities of enzymes in the serum (Schwartz, 1973). These include, (i) over production of enzyme by the tumour, (ii) Tumour blockage of the duct system through which enzyme passes, (iii) induction of enzymes by the presence of tumour, (iv) change in permeability of cell. The enzymes in serum can be divided into two classes viz., (i) metabolically involved enzymes which include isomerases, dehydrogenases and transferases, and (ii) the organ specific enzymes such as amylase, acid and alkaline phosphatase, 5'-nucleotidase, leucine aminopeptidase and γ-glutamyl transpeptidase. The elevated activity of various enzymes have been extensively studied in patients with cancer (Bodansky, 1965).

In the present work, several enzymes including alkaline phosphatase, placental like alkaline phosphatase, lactate dehydrogenase and phosphohexose isomerase have been evaluated as
biochemical markers for detection of lung and oral cavity malignancies.

ALKALINE PHOSPHATASE (ALP) AND PLACENTAL LIKE ALKALINE PHOSPHATASE (PLAP) (REGAN ISoenzyme):

Serum alkaline phosphatase (ALP; EC 3.1.3.1) is a glycoprotein in nature, possessing terminal sialic acid (Fishman, 1974). ALP activity of serum has been separated into various fractions by means of electrophoresis on different media. The ALP activity has been thought to arise from various tissues. They are from (i) placenta, (ii) liver, (iii) other tissues like bones, neutrophils, kidney and intestine (Sussman et al., 1968). Osteoblasts are possible source of ALP to plasma. They secrete the enzyme into circumambient fluid for the participation in extracellular bone formation (Searcy, 1969). The total number of osteoblast in the body constitutes the mass of a sizable organ and could furnish a substantial quantity of enzyme to the blood stream. Tissue ALP also exhibit a distinctive electrophoretic behaviour, indicating that normal human serum contains largely ALP of hepatic origin. ALP presumably derived from the intestine is only observed occasionally in serum, whereas the bone enzyme is rarely found in the blood stream. Earlier studies suggested that to some extent ribonucleic acid synthesis may be regulated by ALP activity and there has been many examples of induction of ALP 'in vivo', some of which require 'de novo' protein synthesis (induction) (Rubini, 1963). ALP attacks various phosphorylated
phenols, alcohols and sugars in two different ways in 'in vitro' system. They either catalyze a hydrolytic cleavage, liberating inorganic orthophosphate or they promote transphosphorylation to an appropriate acceptor without intermediate formation of inorganic phosphate.

Serum ALP assays have been extremely useful in the evaluation of disease of bone (Schwartz, 1973). The patients with osteogenic sarcoma, parathyroid adenoma or cancer metastatic to bone have elevations in ALP activity than the upper limit of normal value (Schwartz, 1976). Elevations in ALP activity have been observed in patients with osteoblastic metastatic lesions, whereas values of ALP are often seen within normal limits in patients with osteolytic disease (Schwartz, 1976). For this reason a greater percentage of patients with prostate cancer metastatic to bone have elevations in ALP than the patients with breast cancer whose bone metastases are most often osteolytic in nature (Schwartz, 1973; Wajasman et al., 1978). There was an excellent correlation between a decrease in total ALP activity and bone ALP isoenzymes in patients who showed good response to anticancer treatment (Rosen et al., 1975). The results indicated that the enzyme activity is sensitive to therapeutic response and the rises and falls in ALP correlated with clinical status of the patients with osteogenic sarcoma.

It is also reported that the elevations in ALP activities are directly proportional to extent of the disease in cancer.
patients with liver metastases (Schwartz, 1976). Viot et al. (1983) have reported that alpha-1-isoenzyme of ALP could serve as biological indicator for hepatic carcinoma. An occurrence of atypical ALP (biliary) in primary liver cancer has been shown by Burlina et al. (1983). Berruti et al. (1993) have considered total ALP monitoring to provide an indication of response, long before radiological evidences in breast cancer patients with osteolytic and mixed bone metastases. Serum ALP can be used to confirm the presence of the cancer or in staging of the disease or to monitor the presence of metastases (Schwartz, 1989). Patients with early Hodgkins disease occasionally show abnormal ALP levels (Johnson et al., 1974).

During the past several years, the use of ALP isoenzyme measurements has been of interest in patients with cancer and a number of variants have been described (Schwartz, 1989). The best known is, placental like alkaline phosphatase (PLAP). Carcinoplaclentale enzyme that has been most extensively studied is called 'Regan Isoenzyme' also. It resembles placental phosphatase in both its physical and catalytic properties. Regan Isoenzyme, one of the oncofoetal antigens, was reported by Fishman et al. in 1968a. He demonstrated the presence of heat stable PLAP in the circulating blood of a 32-year male (P. Regan) with disseminated bronchiogenic carcinoma. The placental type of ALP was named 'Regan' after the name of the patient with lung cancer. The term "carcinoplacental isoenzyme antigen" has been coined because tests showed immunologic identity between
Regan isoenzyme and placental ALP (Fishman, 1974).

Human PLAP (EC 3.1.3.1) is also a glycoprotein in nature. It is indistinguishable from the placental isoenzyme of ALP in the properties such as (i) heat stability (ii) inhibition by L-phenylalanine (iii) electrophoretic migration (iv) action of neuraminidase (v) optimum pH (vi) Michaelis constant (vii) exclusion of antigen-antibody complexes from starch gel during electrophoresis (viii) Ouchterlony plate double diffusion, and (ix) the effect of certain enzyme inhibitors (Fishman, 1974). Furthermore, unlike the liver, bone, kidney and intestinal form of ALP, which are probably encoded at separate genetic loci, PLAP expresses extensive genetic polymorphism and is synthesized in the placental syncytiotrophoblast after the 12th week of pregnancy (Doellgost and Bernirschke, 1979; Fishman et al., 1976). However, it has also been identified as oncodevelopmental protein in various cancer tissues (McLaughin et al., 1982; Yaswen et al., 1985).

Since PLAP is originally reported as a marker in the serum and tumour tissue of a patient with lung carcinoma, the enzyme naturally assumes importance in the diagnosis and management of lung cancer patients. Elevated PLAP activities have also been reported in normal population of smokers and in patients with several non-malignant diseases (Maslow et al., 1983; Muensch et al., 1986; Nathanson et al., 1971; Nielsen et al., 1990; Tonik et al., 1983). However, despite of its low specificity, several investigators have confirmed the potential of PLAP as a tumour
marker in various malignancies (Chen and Hsu, 1985; Eerdekens et al., 1985; Nouwen et al., 1985; Pandit et al., 1988; Tholander et al., 1990; Vergote et al., 1992; Yamamoto et al., 1993). Muensch et al. (1986) have found elevation in PLAP activity in lung, gastrointestinal and ovarian carcinoma. Increased frequency of ectopic production of this enzyme has been reported in patients with malignancy of large intestine or breast (Stolbach et al., 1969). Elevations in PLAP enzyme in patients with various malignancies including lung have been reported by Nathanson et al. (1971). Further they have demonstrated that approximately 14% of cancer patients had detectable PLAP activities. Serum PLAP levels appeared to reflect tumour burden most significantly in patients with ovarian cancer (Chen and Hsu, 1985; Kellen et al., 1976). Using polyclonal antisera, Uchida et al. (1981) have detected the presence of substantial amount of PLAP in tumour tissues and in sera of testicular cancer patients. PLAP has been suggested as potential marker for seminoma (Debroe and Pollet, 1988; Epenetos et al., 1985). Yamamoto et al. (1993) have detected PLAP-like substances in histopathological specimens of seminomas.

**GLYCOLYTIC ENZYMES:**

The glycolytic pathway includes a number of enzymes. Almost all of these enzymes have been estimated in the serum. Warburg (1956) have reported excessive glycolysis as a characteristic of neoplastic tissue. A number of subsequent reports have confirmed the elevated levels of several of these
enzymes especially LDH and PHI in cancer patients (Bodansky, 1954; Rose et al., 1961; Schwartz, 1976).

**LACTATE DEHYDROGENASE (LDH):**

LDH is found in all mammalian cells. It is at high concentration in hepatic, renal and erythroid tissues and also in cardiac and skeletal muscle. LDH, (L-lactate NAD⁺ oxidoreductase: EC 1.1.1.27) is an intracellular enzyme with a molecular weight of 140,000. LDH catalyzes the reversible conversion of lactate to pyruvate with nicotinamide adenine dinucleotide (NAD⁺) as a coenzyme. Multiple molecular forms exist as tetramers composed of various combinations of two subunits designated H (Heart) and M (Muscle). Molecular forms of LDH are easily separated by gel electrophoresis and classified according to their electrophoretic mobility. Malignant cells have distinctive type of metabolism in which glycolytic sequences of tetracarboxylic acid cycle are poorly integrated. Hence, the cells tend to utilize around five times higher glucose than the normal one, most of which is converted into lactate (Lehninger, 1977). Hill and Levi (1954) described elevated serum levels of LDH in patients with neoplastic diseases.

Serum LDH reflects tumour burden as well as tumour growth and/or regression in patients with solid malignancies (Ratcliff et al., 1971; Wood et al., 1973). It has been employed in the evaluation of solid tumours (Malhotra et al., 1986). Rotenberg et al. (1988) have determined LDH and its isoenzymes; in
patients with non-small-cell lung cancer. They have concluded that total LDH in serum might be a direct indicator of clinical stage and tumour burden. LDH activity have been found to increase in patients with primary ovarian carcinoma (Kikuchi et al., 1991). In patients with unresectable colorectal carcinoma with involvement of the liver, serum LDH levels are reported as significant prognostic indicator (Chung et al., 1989). Serum LDH levels are found to be important in the management of patients with malignant germ cell tumours (Schwartz and Morris, 1988). LDH, the beta subunit of chorionic gonadotropin (β-hcG), as well as the number of sites of metastatic disease, when used in multivariate equation, predict the remission in testicular cancer (Bosl et al., 1983). Pressley et al. (1992) have reported the utility of serial LDH measurements in detecting disease recurrence in patients with ovarian dysgerminoma. Brereton et al. (1975) have mentioned pretreatment serum LDH levels as a prognostic indicator in Ewing’s sarcoma. Gluabiger et al. (1980) have studied 76 patients with non-metastatic Ewing’s sarcoma, treated with a variety of regimes, and followed them for minimum one year. Using the Cox’s multivariate life table analysis, LDH revealed strongest inverse association with the disease free-survival. Urinary LDH levels have also been proposed as a marker for carcinoma of the bladder and kidney. However, elevations of this enzyme more closely reflect the presence of pyuria, hematuria, or bacteriouria, hence its use in these context has
been discouraged (Fleisher et al., 1981). In case of melanoma 72% of patients with elevated serum LDH levels had recurrence of their tumours, compared to 3% of those whose LDH levels remained normal (Fink et al., 1983). The main site of metastases in patients with elevated LDH levels was the liver. Another report (Copur et al., 1989) advocated serum LDH activity as prognostic indicator in patients with multiple myeloma. Cohen et al. (1981) reported a significant correlation between higher LDH activity and a worse prognosis in small-cell lung cancer. Aroney et al. (1984) reported significant correlation between disease status and serum LDH levels in patients with small cell and non-small cell lung cancer. Increased LDH activity have been reported in sera of patients with leukemias, lymphomas and other haematological disorders (Bosl et al., 1991; Buchsbaum et al., 1991; Flanagan et al., 1989; Pandit et al., 1990; Patel et al., 1991). Above cited literature strongly supports the usefulness of LDH determinations as diagnostic aids in patients with cancer.

**PHOSPHOHEXOSE ISOMERASE (PHI):**

Phosphohexose isomerase, or glucose-6-phosphate isomerase (PHI: EC 5.3.1.9), is an enzyme that catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate. PHI is widely distributed in human tissues and is abundantly found in liver, skeletal muscle, bone, brain and lung (Bodansky, 1954). From studies on human PHI from erythrocytes and muscle tissue as well as from genetic analysis it is known to consist
of two identical subunits with a molecular weight of approximately 60,000 (Mohrenweiser, 1987; Tsubio et al., 1971). Joplin et al. (1962) reported decreased levels of serum PHI in clinically responded cases of breast cancer. Bodansky (1954) described a method for determining PHI in blood and found higher activity in the sera of patients with cancer. The relatively high sensitivity of PHI in lung cancer was reported by West and his associates (1962). Schwartz et al. (1985) have also reported clinical significance of PHI as marker in lung cancer. They found PHI, to be a valuable diagnostic tool for lung cancer patients. The results of Santabarbara et al. (1988) have indicated prognostic significance of PHI in patients with non-small cell lung cancer. Baumann et al. (1988) have observed a general rise in PHI and other glycolytic enzyme activities in the malignant tissue in early stages without metastases and in well differentiated tumours. There are many other reports dealing with the clinical significance of PHI in tumour diagnosis and especially for the follow-up of cancer patients (Das et al., 1985; Filella et al., 1991; Roguljic et al., 1977; Schwartz et al., 1976). Utility of serum PHI as an useful indicator in the preventive checkup of gastrointestinal and renal cancer in medical practice has also been reported (Baumann et al., 1990).

Based on their observation on expression of PHI activity by human neuroleukin cDNAs when transferred to monkey COS cells (CV, origin SV 40), Chaput et al. (1988) and Faik et al. (1988)
reported that PHI exhibits strikingly high degree of homology to the neurotrophic factor neuroleukin. According to the Baumann and his associates (1988) PHI or a very closely related derivative endowed not only with glycolytic activity but also as trophic factor related to proliferation and malignancy. Serial determinations of serum PHI are valuable in following the course of the malignant disease during treatment (Damle et al., 1971a; Das et al., 1985; Rao et al., 1976; Yeshowardhan et al., 1986). As evidenced from the previous literature, serum PHI as biochemical marker has become an area of contemporary interest for oncobiochemist in diagnosis as well as prognosis of various malignancies.