2.0 INTRODUCTION

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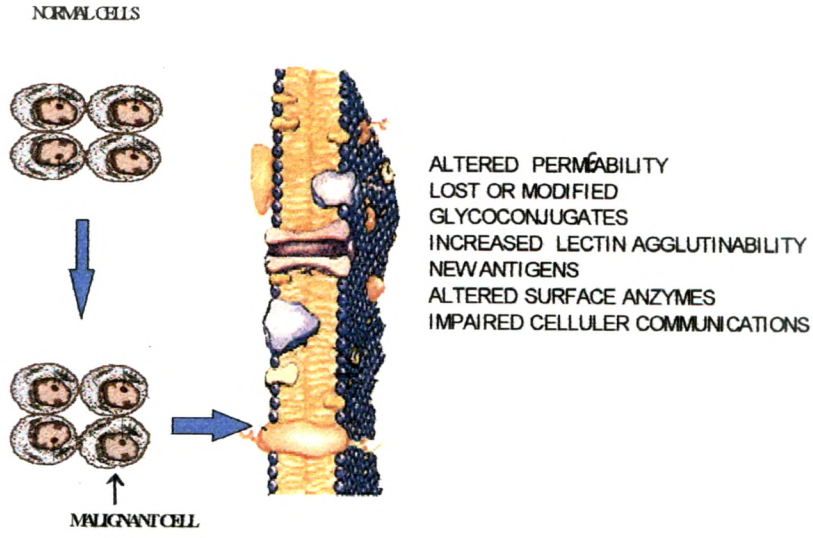
2.1 GLYCOPROTEINS AND CANCER

Figure-1
CELLSURFACE CHANGES DURING MALIGNANT TRANSFORMATION



Cancer is a major cause of morbidity and mortality world-wide. This dreadful disease has remained one of the serious health problems for the modern world. Malignant neoplasm can be regarded as a caricature of certain normal biological processes because profound alterations occur in the morphology, metabolism and biochemical composition of cells during malignant transformation. Malignant neoplastic cell has different characteristics so as to become "asocial" to their environment including neighbouring cells. During malignant transformation, normal cell undergoes biological variations, which leads to an apparent failure of normal growth control mechanisms. The external factors that determine a cell's pattern of growth like growth stimulating components must first impinge on the cell surface. The cell surface harbours normal physiological properties related to neoplastic

transformation and metastatic spread of disease including cell shape, growth, cell division, differentiation, cellular recognition, communication, adhesiveness, migration and contact inhibition of growth and immunological competence (Alhadeff, 1989). It is known that a fundamental defect of tumour cell could reside in the cell membrane. However, biological significance of various membrane changes for malignant growth is still poorly understood (Smets and Vanbeek, 1984). Glycoconjugates, the carbohydrate conjugated biomolecules are now recognised as important biomolecules localised in various cell membranes (Kornfeld and Kornfeld, 1980). Malignant transformation associated carbohydrate alterations were first discovered in cultured cells by Hakomari and Mirakami (1968). Since this first report, there was an overall increase in cross peak attributable to altered cell surface of glycoconjugates in carcinoma when compared to non-transformed progenitor cells (Fukuda, 1994; Nicolson, 1976; Yogeeswaran, 1983). In spite of their relatively small proportion by weight, the surface carbohydrates cover entire cell surface (Jarnefelt et al, 1978). The carbohydrate moieties of membrane glycoconjugates contribute to important cell surface characteristics demonstrated by malignant cells (Hakomori, 1989; Yogeeswaran, 1983).

Alterations of cell surface have been found to severely affect the metastatic potentials of experimental tumours (Hakomori, 1989). The cell surface carbohydrate interactions in metastatic cascade have been therapeutically envisaged in following three steps: **(i)** release of tumour cells from primary tumour mass due to altered homotype adhesion phenomena, **(ii)** the mechanism of blood transportation of metastatic tumour cells by heterotypic cell interactions and **(iii)** the arrest of cells in an organ by specific interactions with the target tissue. Important involvement of carbohydrates in metastatic formation would imply that tumour cells with different metastatic potentials possess distinct qualitative or quantitative differences in their glycosylation of glycoconjugates.

A biological membrane is a lipid bilayer containing embedded proteins, mainly glycoproteins. The later components have been likened to iceberg floating in

a lipid sea and their carbohydrate moieties are accessible on the exterior of cell. The biological advantage of addition of oligosaccharides to glycoproteins is not fully understood. The biological functions of glycoproteins are well established, however, the role of carbohydrate moieties in these functions is largely speculative (Olden et al, 1982). The carbohydrate residues are believed to work as: **(i)** starting signals for directing glycoproteins to specific cellular organelles and tissues (Hasilik, 1981) **(ii)** protector of glycoproteins from proteolytic degradation (Olden et al, 1982). Striking differences between the carbohydrate structure found on normal and malignant cells have been reported by various workers (Feizi and Childs, 1987; Hakomori, 1989; Yogeessawaran, 1983).

2.2 ALTERATIONS IN GLYCOPROTEIN CONSTITUENTS IN MALIGNANCY

On the one hand carbohydrate epitops act as antigens e.g. differentiation and oncodevelopmental antigens (Feizi, 1981) and on other hand they can mask antigenic sites on glycoconjugates and thus can represent "Anti – Antigen" (Schauer and Corfield, 1982). Although, over 100 different monosaccharides are found in various biological systems only about seven monosaccharides account for almost all the carbohydrate residues in mammalian cell glycoproteins. Occupancy at terminal or near to terminal position underlies the vital role of these sugars in determining surface characteristics of cells and secreted glycoproteins (Schauer et al, 1995).

In the instances, sialic acid and L-fucose occupy a terminal position at the non-reducing end of oligosacarides. Being non-reducing terminies sialic acid and fucose have attracted more interest and has gained outstanding importance, (Flowers, 1981; Vangsted, et al, 1994). In human, sialic acid appears in its most common form, N-acetyl neuraminic acid (NANA). Sialic acid, an acetylated derivative of neuraminic acid is widely distributed in mammals (Schauer et al,1993). Being a terminal sugar, it plays a major role in the chemical and biological diversity of

glycoconjugates and cell type specific expression of glycosyl transferases (Paulson, 1989). Specific sialylation pattern of oligosaccharide can be considered as key determinants in the make up of cells. Sialic acid has a strong negative electrical charge which influence the spatial configuration of sialic acid containing molecules (Schauer and Corfield, 1982). On their surface, tumour cells carry a great amount of sialic acid, which is responsible for negative charge of malignant cells. Elevated cell surface sialic acid has been shown to correlate with spontaneous metastatic potentials and invasiveness (Collard et al, 1986; Passaniti and Hart, 1988). The idea of using sialic acid as a marker for malignancy goes back to Skipski et al (1971), who have described a protein-lipid complex neoproteolipid-ω in Walker carcinoma and neoproteolipid-s in man with hepatoma and other tumours. Sialic acid a membrane constituent is assumed to enter the circulation in various ways like shedding, cell lysis etc. The serum concentrations of sialic acid depend not only on the concentrations of glycoproteins, but a substantial part of the serum sialic acid is also bound to acute phase reactants. It therefore reflects cytokine induced changes in the biosynthesis and post-translational glycosylation processes of sialoproteins in liver (Pos et al, 1990; Van Dijk et al, 1991). Earlier reports on alterations in the sialic acid in cancer patients have stimulated the interest in these components as possible tumour markers (Bhuvarahamurthy et al, 1995; Painbeni et al, 1997; Shashikanta and Rao, 1994; Verozin et al, 1990). Subsequently, various workers have examined importance of alterations in sialic acid levels in diagnosis and management of cancer patients (Feijoo et al, 1997; Paskowaska et al, 1998; Romppanen et al, 1997).

Besides sialic acid, another monosaccharide found at nonreducing terminal in the chitobiose structure of the oligosaccharide chain of glycoproteins is fucose. According to Neuberger et al (1966), fucose is one of the most common sugars associated with glycoproteins. L-fucose is methyl pentose normally present at low concentrations in serum and is the only levorotatory sugar utilised by mammalian system. The metabolism of L-fucose is only

partially understood. Any change in the levels of cell-surface glycoproteins would be accompanied by alterations of the fucose levels (Listinsky et al, 1998). Numerous studies have shown that malignant changes may be accompanied by increased expression of membrane associated fucose containing macromolecules (Glick, 1978; King et al, 1980). There is abundant evidence, which suggest that the fucose metabolism is disturbed in cancer. (Aoyagi et al, 1993; Hakomori, 1989; Thompson et al, 1992). Several investigators have provided evidences for altered serum fucose values in breast cancer, lung cancer and various other malignancies (Baxi et al, 1991; Turner et al, 1985). A positive correlation with progression of tumour growth rate in mice bearing transplanted tumours have been reported by Sen and Roychowdhury (1984). A positive correlation has also been found between the stage of the disease and magnitude of elevations in serum fucose levels (Gosh and Nayak, 1991). It is documented that the alterations in L-fucose values can be utilised to estimate the extent of malignant disease as well as for analysing the outcome of anticancer treatment in various malignancies. Sakai et al, (1990) have observed high excretion of fucose through urine in cancer patients than that in patients with benign diseases or healthy individuals. An overall increase in crosspeak attributable to cell surface fucosylation in carcinoma as compared to benign lesions has been reported by Miliotes et al (1996). A change in the serum,focussed values in relation to progression and regression of tumour growth rates has focussed attention on its possible use as an index of experimental tumour growth (Fernandez et al, 1997; Wang et al, 1995). Tumour can shed fucose containing macromolecules into their environment and may also contribute indirectly to serum fucose by promoting increased fucosylation of existing glycoproteins. Low fucose incorporation in cell surface carbohydrates was found to be associated with inhibition of invasion (Bloscher et al, 1989). All investigators are unanimous in recording higher levels of fucose during malignancy irrespective of the lesions and organs involved. A significant relationship of sialic acid and fucose with the biological factors of glycoproteins has been reported (Dische and Shettles, 1963). The relative proportions of sialic acid and fucose differ significantly in different serum

glycoproteins. The ratio of one to another may give information on the nature of glycoprotein alterations in specific conditions (Winzler, 1955). Elevations in carbohydrate containing proteins have been considered to be a reflection of disturbed integrity of the glycosylation of neoplastic cells (Laganana et al, 1998; Verozin et al, 1990).

The precise mechanism of raised levels of sialic acid and fucose is still obscure and different hypothesis have been advanced. These include: **(i)** increased glycosylation of serum glycoproteins, **(ii)** shedding of glycoproteins from tumour cell surface, and **(iii)** increased concentrations of normal serum proteins (Turner et al, 1985). The possibility to account for increase in serum fucose contents may be due to depolymerization of ground substances of the connective tissues or surgical trauma, severe inflammation and tissue necrosis or may be hyperplasia of cells (Tatsumura et al, 1977).

Increased levels of monosaccarides other than sialic acid and fucose (like mannose and galactose) are reported in several types of transformed cells and solid tumours (Warren et al, 1978). Increased levels of carbohydrates in the fraction remaining after precipitation with ethanol is a more sensitive marker of neoplastic and other diseases (Winzler, 1955). Seromucoid fractions are a complex mixture of many different proteins, which are perchloric acid soluble and phosphotungstic acid precipitable fractions (Thaw and Albutt, 1980; Varley et al, 1984). The protein components of mucoids or mucoproteins are combined with large amount of carbohydrates. The carbohydrate portions of the conjugated proteins are complex substances. Analysis of seromucoid fractions can be made in form of its basic constituents i.e. protein and carbohydrate contents. Around 10 % of total protein bound hexoses of the human sera are of seromucoid fractions; therefore, it is generally measured in terms of hexoses and protein (mucoid protein) contents. The elevations in seromucoid fraction have been reported as suggestive of the presence of malignant tumours (Van-Dink et.al, 1994). Various investigators have determined hexoses levels and reported that elevated levels of hexoses are

associated with metastatic behaviour (Macbeth and Bekeshi, 1962) as well as poor treatment outcome (Rossato and Ravdin, 1968). The measurement of seromucoid fractions are found to be useful in assessing tumour burden and immune reactivity (Bradley et al, 1977). The hexoses levels alone are reported to be far more reliable marker for detection of malignancy than in combination with acute phase reactant proteins (Walker and Gray, 1983). Furthermore, the authors have reported its clinical role in assessing the extent of response to therapy in patients with colon and rectum cancer. Evans et al (1974), have reported significance of serum protein bound neutral hexoses for estimation of malignant tumour extension and in monitoring the efficacy of therapy. Elevated levels of serum protein bound carbohydrates in patients with malignant neoplasia have been investigated by various workers (Smets and Vanbeek, 1984; Yogeeswaran, 1983). The reports have suggested that the elevations in serum hexose content are possible indicator of dissemination of malignant disease and treatment out come.

2.3 ALTERATIONS IN GLYCOPROTEIN ELECTROPHORETIC PATTERNS

Probably the major characteristic feature associated with malignancy is the enrichment of glycopeptides in transformed cells when compared with their normal counter parts irrespective of species, cell type and transforming agents (Atkinson and Bramwell, 1981; Smets and van Beek, 1984; Warren et al, 1978). Continuing research of circulating markers for neoplasia has yielded many tumour markers, most of them are glycoprotein in nature.

As detailed in **table-1** several glycoprotein markers are reported to have significant clinical value for cancer patients. However, usefulness of number of circulating tumour markers and tumour associated antigens extensively used in cancer patients is still being debated and no conclusive data are available. Different glycoproteins in serum represent a sensitive reflection of the physiological and biochemical conditions of the

body. The appearance of different glycoforms of proteins appears to be a sensitive at the time of their synthesis and release (Rademacher, 1988).

Table – 1

GLYCOCONJUGATE TUMOUR MARKERS

Glycoconjugate	Description
Carcinoembryonic antigen	Oncofetal glycoprotein
Alpha-feto protein	Oncofetal glycoprotein
Heat-stable alkaline phosphatase	Oncofetal glycoprotein
Epiglycanin	Mucustype glycoprotein
Mammary tumour glycoprotein	Glycoprotein
CA-549	Glycoprotein
CA-125	Glycoprotein
Alpha-1 acid glycoprotein	Serum glycoprotein
Alpha-1 antitrypsin	Serum glycoprotein
Gamma-glutamyltranspeptidase	Glycoprotein
Circulating immune complexes	IgG-Fucoglycolipid
P-glycoprotein	Glycoprotein
TAG-72	Sialoprotein
Bone sialo protein	Sialoprotein
Haptoglobin	Glycoprotein

Serum protein electrophoresis is widely used as a diagnostic tool for various pathological conditions including malignancies (Macbeth and Bekesi, 1962). Using electrophoresis, serum proteins can be resolved into the albumin and three globulin zones designated as alpha, beta and gamma, respectively in

decreasing order of their mobility. This method has made it clear that the globulin fraction of serum proteins also represent a group of proteins. Thus, protein electrophoresis can provide a comprehensive view on a large number of proteins simultaneously. Glycoproteins as a group have multiple and complex functions and are found as cell surface receptors, surface antigens, transport proteins, enzymes, hormones and blood group substances. Because of important role of glycoproteins in cancer, their constitutes are selectivity associated with malignant conditions. This has remained the reason forever increasing clinical value of this subject as tumour markers (Bates, 1991; Consensus Report, 1987; Sell, 1993). The rate of protein synthesis in growing diploid human fibroblast was found to be higher than in rest of the cultures (Ruoslahti et al, 1973). The rate of carbohydrate synthesis was found to be four times higher in growing cells than in non-growing control cells (Kaplan and Moskowitz, 1963). Increased expression of high molecular weight glycoproteins occurring with malignancy has been reported earlier (Rye and Walker, 1989; Smets et al, 1977). There was a higher incidence of acidic glycoprotein in region of 210-280 kDa in malignant tissue than the benign (Rye and Walker, 1989). Increased expression of 130 k Da and decreased expression of 55 k Da of glycoprotein found in malignant tumours as compared to benign diseases. Dermer et al, (1987) have identified the band at 47 k Da from benign and malignant tissues that showed significant alterations in cancer patients. Five different glycoprotein bands were also detected more frequently in squamous cell carcinoma (O'Brien et al, 1991). Over-expression of 95 k Da membrane protein in cancer cell has also been reported (Chen and Magalhaes, 1990; Doyle et al, 1995). The literature on alterations in glycoproteins during cancer indicates that precise evaluation of changes in glycoprotein electrophoretic pattern can reveal significantly useful information. It would be more informative to check qualitative changes in serum glycoprotein. Consequently, it will not be unreasonable to anticipate that serum glycoprotein electrophoretic patterns can be of significant clinical value in diagnosis and management of cancer patients.

2.4 ASSOCIATION OF CHANGES IN SIALOPROTEINS AND FUCOPROTEINS WITH CANCER

The lectins are multivalent saccharide binding proteins. These glycoproteins of non-immune origin can agglutinate cells and precipitate glycoconjugates (Lis and Sharon, 1986). Therefore, these plant products are widely used for identification of specific sugar residues. It has specific affinity for selective linkage and thus binds to those sugar moieties only. Affinity to specific lectin can provide information on altered glycosylation patterns that are believed to be associated with malignancy. The lectins show specificity for a wide range of different sugars or sugar sequences. Therefore, they provide the means to identify the structural features of carbohydrate chains of glycoproteins (Lis and Sharon, 1986). *In situ* applications of lectins have provided information about the identity and distribution of specific glycoconjugates (Schachter, 1986).

The lectin probes show considerable potentials for studying glycoproteins in secretions and in their cell of origin (Feijoo, 1997). Lectin binding studies have provided evidences suggesting that carbohydrate moieties of glycoproteins are modified during malignancy (Turner, 1992). Structural analysis of the tumour associated carbohydrate antigens have shown that sialylated and fucosylated oligosaccharide derivatives together with some related structures may essentially be associated with malignant transformation (Holmes et al, 1986; Nuddleman et al, 1986). Previous studies using lectins have reported that aberrant glycosylation expressed in human cancer can define stage, direction and the fate of tumour progression (Dall'olio and Trere, 1993; Mustafapour and Goldstein, 1993). Previous histochemical studies using lectins have also confirmed that there are differences in specific sugar groups between glycoproteins of benign and malignant tissues. Some of the glycoprotein alterations were found related to tumour differentiation or to metastatic potentials (Rye and Walker, 1989).

Earlier investigations in cancer patients consistently showed abnormal levels of normally occurring proteins (Macbeth and Bekesi, 1962). Terminal epitopes of carbohydrates have been proposed to play significant role in cell-cell interactions, in development of cell adhesion and in malignant transformation (Domino et al, 1997). One of the most common changes in cell surface carbohydrates during malignant transformation is the increase in size of oligosaccharides resulting in branching sites for incorporation of sialic acid (Harvey et al, 1992). The role of specific linkage of sialic acid in the development of an invasive phenotype in cancer have been suggested by several investigators, but, it has never been conclusively demonstrated. The extent of sialylation found in tumour cell surface is claimed to be related to the metastatic potentials of the tumours (Yogeeswaran, 1981). The expression of sialyl glycoconjugate changes during development differentiation and oncogenic transformation have been reported earlier (Hakomari, 1981). It is found that the information regarding alterations in sialoglycoproteins may be useful in differentiation of patients with malignant disease as well as to predict treatment outcome (Marth et al, 1988; Ogoshi et al, 1997; Shamberger, 1984; Stefanelli et al, 1985; Verozin et al, 1990). It has been suggested that reduced sialylation of tumour cell surface antigens may prevent implantation into distinct sites resulting in a reduced incidence of metastasis (Harvey et al, 1992). The expression of alpha 2-6 sialylated sugar chain is remarkably increased in the majority of the colon cancer tissues (Gessner et al, 1993). Increased levels of membrane alpha 2-6 sialylation appears to be related with more invasive behaviour of cancer cells (Dall'olio and Trere, 1993). Sambucus Nigara is the lectin that specifically binds to sialic acid attached via alpha 2-6 linkage (Sata et al, 1991). Lotus tetragonalobus is a plant lectin which specifically binds to fucose, thus, used for recognition of fucosylated constituents (Periera and Kabat, 1974). The functional significance of expression of fucosylated structure in tumour cells is unknown, however, this epitope has been recognised as binding ligand for selectine molecules (Bradly et al, 1977). Markedly elevated expression of fucosylated glycoconjugates has been reported in sera of cancer patients (Turner et al, 1995; Ayoyagi et al, 1993; Kondo et al, 1994). Information

regarding glycosylation index of abnormal proteins provide a potent criterion for early diagnosis of neoplastic diseases of liver (Ayoyagi et al, 1988). Differentiation of mammalian cells can be accompanied by structural changes in fucosylated surface carbohydrates (Hakomori, 1989; Vangsted et al, 1994; Zieske,1982). Previous studies using lectins confirms that there are differences in specific sugar groups between the glycoproteins of benign and malignant tumours and some alterations are related to tumour differentiation (Walker and Gray, 1983). The reports have suggested that in-depth studies are essential to provide clinically useful data.

2.5 CHANGES IN THE ENZYMES OF GLYCOPROTEIN METABOLISM

The origin of variety of sugars that facilitate the fine-tuning of the surface properties to fulfil specific biological functions has been the subject of much interest. If the enzymatic steps are identified, a comparative study may help to relate these special end products to the physiology of concerned cells. The study of association of the enzymes involved in glycoprotein metabolism with malignancy can be a step forwards in understanding the relationship between malignant transformation and alterations in glycoproteins. The mechanisms of regulation of sialic acid and fucose levels upon malignant transformation can be studied by the enzymes involved in anabolism as well as catabolism of glycoproteins containing these sugars at termini. To gain insight into potential modifications of serum glycoproteins, study of glycoprotein modifying enzymes should be useful. The enzymes modify it either by addition of sugar residue or by removal of terminal residue of sugar. The glycosylation and deglycosylation are the addition or removal of sugar moieties of glycoproteins to or from adequate acceptors, respectively. A group of cellular enzymes called glycosyl transferases and glycosidases are responsible for sequential addition and deletion of monosaccharides to appropriate proteins. The glycosidases cleave monosacharide units from the nonreducing end of oligosaccharides. These enzymes have two kinds of specificity, the glycan

specificity and the glycan specificity towards their substrate. Endoglycosidases, which release monosaccharides from glycoproteins and glycolipids, have opened a new age in structural study of glycoconjugates. The desialylation of the sialoproteins and defucosylation of fucoproteins can be quantified by sialidase and fucosidase activity, respectively. A study of various glycosidases in human colon and breast cancer showed marked higher values in malignant tissues. Significant decrease in levels of glycosidase activity was reported in sera of cancer patients. β-hexosaminidase and α-L fucosidase have a diagnostic significance because it has been described to be altered in several diseases (Deugnier et al,1984). The α-L flucosidase cleaves terminal fucose residue from glycoconjugates .It is ubiquitously found in human tissues and extra cellular fluids (Durnand et al, 1982). The biochemical mechanisms of determining levels of α-L fucosidase in human serum are unknown. Previous evidences have suggested that low α-L fucosidase activity might be a hereditary condition associated with increased risk of cancer (Barlow et al, 1981). Increased fucosidase activity in tumour groups as compared to normal tissues has also been reported (Wang et al, 1995). In the studies of high and low metastatic cell lines, changes in fucose metabolism have been noted (Dennis and Kerbal, 1981; Finne et al, 1980; Schwartz et al, 1984).

Sialidase enzymes recognise and cleave terminal sialic acid from glycoconjugates, when compared to bacterial or viral sialidase. Eukaryotic sialidase is expressed at lower levels and frequently show poor expression. Sialidase is located in lysosomes and plasma membrane. Metastatic potentials of malignant cells are inversely proportional to lysosomal type of sialidase activity (Miyagi, 1994). It is reported that cancer patients had lower levels of neuraminidase as compared to that in healthy individuals (Rothenberg et al, 1994). The authors have further documented that deficiency of neuraminidase may suggest an elevated risk for cancer (Rothenburg et al, 1996).

It is estimated that over 100 glycosyl transferases are needed to account for

the synthesis of known carbohydrate structure. A regulated expression of these enzymes is generally believed to account for the synthesis of specific cell type carbohydrate structure. Each glycosylating transferase is specific for both the nucleoside diphosphate sugar and the amino acid residue of the protein. The numbers of such transferases in mammalian cells and their speficities involve considerably more of the structure of the glycoproteins. One of the mechanisms for appearance of these enzymes in serum may be by the shedding of the plasma membrane constituents into systemic circulation of the host (Black, 1980). The specific linkages of terminal sugars to the oligosaccharide backbone have a major role in the organ specific immuno determination. Thus, the family of enzymes that catalyzes transfer of terminal sugar residues of glycoproteins and glycolipids play a crucial role in malignant transformation. In general, glycosyl transferases are membrane bound enzymes but soluble forms have been found in human physiological fluids (Ronquist and Nou, 1983). Altered glycosyl transferase activities in malignant cells and tissues as well as in sera of cancer patients have been reported (Mas et al, 1998; Wang et al, 1995).

The extent of sialylation and fucosylation of the glycoproteins and glycolipids are determined by looking at the sialyl transferase and fucosyl transferase activities. Sialyl transferase catalyses incorporation of sialic acid from nucleotide sugar to terminal position of carbohydrate residue. Fucosyl transferase constitute a family of glycosyltransferases, incorporating fucose into glycoprotein and glycolipid glycans (Shetlar, 1961). Thus, sialyl transferase and fucosyl transferase families are characterised by having sialyl moiety and key regulatory enzymes that control the glycoconjugate biosynthesis pathway. Sialyl transferase and fucosyl transferases attribute to the diversity in carbohydrate structure through the attachment of sugar by different linkage in various terminal positions. Elevations in sialyl transferase activities have been reported in tissue and sera of cancer patients (Lileng, 1993; Yamamoto et al, 1995; Hada, 1997). Transformed cells exhibit an altered activity of sialyl transferase as compared to normal fibroblast (Basu et al, 1996; Hada, 1997). Selective increase in specific sialyl transferase has been

observed in human cancer (Gessner et al, 1993; Asano et al, 1995). Alpha 2-6 Sialyl transferase acting on glycoprotein acceptor behaves in colonic cells as an oncodevelopmentally regulated enzyme. Increased expression of alpha 2-6 Sialyl transferase was reported in meningioma by Yamamoto (1995). Immunohistochemistry and *in situ* hybridization histochemistry suggested that sialylation by the sialyl transferase is dominant in tumour cells, whereas hydrolysis of sialic acid by sialidase is dominant in apoptotic bodies. The enrichment of glycoprotein by fucose is associated with cancer and enzymes responsible for this is fucosyl transferase. Various workers have documented association of malignant transformation with alterations in fucosyl transferase. Characteristic decrease in the incorporation of the C^{14} fucose into its endogenous acceptor was found after surgery (Bauer et al, 1978). Many of the individuals show increased activities in the blood of fucosyl transferase (Bauer et al, 1978; Chandrashekhran et al, 1992). The enzyme levels are found to correlate with extent of disease (Dao, 1980) and prediction of relapse (Bauer et al, 1978; Wang et al, 1995).

In spite of significant role of the enzymes including sialyl transferase, fucosyl transferase, sialidase and fucosidase very few reports have appeared on their simultaneous alterations in human malignancies; therefore, further work on these enzymes is imperative.

2.6 OBJECTIVES OF THE PRESENT STUDY

An understanding of malignant cell highlights those normal mechanisms that have become deregulated. But, despite significant advances in our understanding of the biology of cancer cells, relatively little has changed on the clinical scene so far. The literature survey indicates that in-depth evaluation of glycoprotein changes can reveal clinically useful information for early diagnosis and better management of cancer patients. Accordingly, the present attempt was made to determine whether glycoprotein constituents could reveal clinically valuable information for early diagnosis and better

management of the breast and oral cavity cancer patients, the major leading sites of cancer in Gujarat state. The goal was to define glycoprotein changes to understand ways to halt the progression from dysplasia to invasive cancer. Therefore, the present study included patients with BBD/OPC. In other two groups of individuals of the study, first population consisted of individuals without any disease and no major illness in recent past. The second population consisted of cancer patients. More than one half of the individuals bearing malignancy are expected to develop recurrence and/or metastasis. Successful implementation to provide a long-term disease free survival to these individuals depends on the ability to identify those asymptomatic individuals who have occult malignant disease or are at an increased risk of recurrence and/or metastasis. The overall results of treatment of cancer reflect the biological behaviour. Therefore, the study included long-term serial follow-up samples of cancer patients after initiation of anticancer treatment. Following objectives were defined so that the present study can be helpful to obtain detailed information about glycoprotein changes which is of utmost importance, because such knowledge can solve many problems relating to cancer care and management.

OBJECTIVES:

- To estimate glycoprotein constituents including different forms of sialic acid, fucose and seromucoid fractions as well as to measure activities of the enzymes including fucosidase, sialyl transferase and fucosyl transferase from sera of healthy individuals, patients with BBD/OPC and cancer patients at diagnosis as well as during follow-up.
- To determine whether these biomolecules are useful as diagnostic tool.
- To correlate levels of the biomarkers with extent of malignant disease and effectiveness of therapy.
- To study association of alterations in glycoprotein constituent levels with the enzyme activities.

- To assess value of the glycoprotein constituents and enzymes as prognosticators for cancer patients.
- To separate serum glycoproteins by electrophoresis and to evaluate the alterations in electrophoretic patterns during malignancy.
- To assess specific glycosylation changes of serum glycoproteins by lectin affinity chromatography in the subjects.
- To evaluate the glycoprotein changes during long-term follow-up in cancer patients.
- To study the glycoprotein changes in malignant and non-malignant tissues as the confirmatory analysis of circulatory alterations.