5.0 DISCUSSION

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5.1 ABOUT THE CONCEPTS

The significant progress that has been made in the prophylaxis and treatment of cancer during the past few decades provides optimism that the war against this formidable foe can be won only if the armamentarium currently used for diagnosis and management is strengthened. The search for tumour markers the only effective weapon to win the battle has occupied many investigations. They share the same sentiments that of how to identify the changes that would harm before they have a chance to act? Unfortunately, although the *Nature* may have done something to malignant cells that makes them distinguishable from normal host cells, most often, the difference between cancer cell and normal host cell is not perceptible to our senses or defences until substantial and often irreparable harm has been done. Majorities of cancers are disseminated at the time of presentation, which makes the management of the disease difficult. The deeply located tumours may not be noted until grown to a large size. Increasing number of patients also seek accurate estimates of their prognosis. The doctors too require these estimates while planning anticancer therapy to have precise informations regarding its effectiveness. In order to evaluate disease status at different time interval during/after anticancer treatment, the investigations need to be repeated frequently. In these circumstances, an approach to establish a simple, non-invasive and reproducible blood-based marker system, which can be helpful in early diagnosis and treatment monitoring of patients with neoplastic diseases, becomes most essential. So strong and pervasive these and other reasons are, that this approach has been continuously proposed and perhaps with limited success newer tests have been made available intermittently.

A common property underlying any successful tumour marker, whether it is to be used for screening, diagnosis and prognosis or monitoring is that its values should differ significantly among clinical subgroups of interest. For

instance, if a marker is being considered for screening, its value should differ significantly between cancer and healthy individuals. If the tumour marker lacks this basic property, it should be discarded from any further clinical evaluation because its discriminatory capabilities are nil. The interest in the field of tumour markers should not only aim at distinguishing between malignant and healthy conditions but, it should also differentiate malignant from non-malignant diseases. The cancer cell grows and divides generally through progressive stages from preneoplastic to malignancy. Therefore, one must investigate marker levels in patients with benign/preneoplastic diseases also. In some instances, association of the markers with cancer is only seen in untreated cancer patients. In such cases, efficiency of study can be greatly enhanced if they are evaluated during/after treatment. Also, differences in therapeutic outcome after anticancer treatment cannot be explained only by clinical findings. These points have provided us the rationale to include following populations in the study: **(i)** healthy individuals (separate groups for two malignancies), **(ii)** patients with BBD/OPC who have increased risk of developing cancer, **(iii)** breast cancer patients and oral cavity cancer patients at the time of diagnosis and **(iv)** cancer patients during/after anticancer treatment. The history of the search for tumour markers is chequered. Usually, some proteins, hormones, enzymes or antigens are analyzed as tumour markers in a smaller number of cancer patients and controls, but not in pathological controls. Initially, high sensitivity and reliability are claimed based on the observations of a smaller number of individuals. But, after several years of initial reports, the results are usually found to be contradictory on larger population. Hence, a study on tumour markers should overcome these pitfalls. Accordingly, the present study analysed large number of blood samples. Further, the science of biostatistics can play a significant role in addressing important features of tumour marker evaluation. By properly applying sound statistical principles to the development and evaluation of tumour markers, one can successfully add useful information to oncologist's weapons while eliminating those with little potential for accurately predicting the patient's health status. Hence, the present study most suitably and precisely used statistical methods including Fischer's exact

and paired "t" test, ROC curves analysis, Kaplan Meier survival curves and Regression analysis.

During past decade, a virtual explosion in the knowledge and understanding of the biology of cancer cells has been emerged. One of the distinguishable features of the neoplastic cells is the cell surface that differs in many respects from its normal counterparts (Fukuda, 1994; Nicolson, 1976; Yogeeswaran, 1983). It is not surprising therefore that several types of neoplastic transformations are accompanied by alterations in the composition of glycoproteins (Bhavnanandan and Davidson, 1982) the major components of cell surface. The greatest significance of glycoproteins is its widely acknowledged role in majority of cell surface functions (Atkinson and Bramwell, 1981; Fukuda, 1994; Nicolson, 1976). By definition, ~~of course,~~ cancer cells have altered cell surface characteristics. Like the investigations before, the present study was started to grapple with the question what specific clinical usefulness can be provided by cancer cell glycoproteins?

Tumour cells seem to shed their surface components more than normal cells. Black (1980) has reviewed the phenomenon of cell surface shedding of molecules, its role in normal membrane protein turn over and its importance in certain disease processes. Shedding of surface components may be mediated by an increase in cell surface glycoproteins (Yogeeswaran, 1983). Since last few years, knowledge and appreciation of the role of glycoproteins and their constituents as markers of cancer have been both increased and refined. The inter-relationship among the alterations in glycoprotein structures and their impact on malignant changes associated with cell morphology are now more clearly established. Considering the view that malignant transformations are associated with alterations in glycoproteins (Warren et al, 1978; Yogeeswaran, 1983), they can't be subdivided on the basis of their constituents. The higher rate of protein synthesis in growing cells, as well as malignant cells are reported (Kaplan and Moskowitz, 1963). Complex cell surface oligosacharides are implicated in numerous biochemical phenomena (Fukuda, 1994; Varki 1993). It is reported that there are two

major functions of carbohydrate moieties of proteins: **(i)** to act as starting signal for directing glycoproteins to specific cellular organelles as well as tissues and **(ii)** to protect glycoproteins from proteolytic enzymes (Olden et al, 1982). Sialic acid and fucose, the important glycoprotein constituents which are typically found at the terminal position on vertebrate oligosaccharides have significant role in clinco-pathological features of cells (Flowers, 1981; Hakomori, 1989; Schauer et, al 1995; Warren et al, 1978). An unusual type of post-translational modifications in which sialic acid or fucose is glycosidically linked to glycoprotein, are found to be elevated in malignant cells(Chandrasekaran and Davidson, 1979;Santer and Glick,1983).

Since evaluation of glycoprotein changes in part may not suffice all clinical requirements, comprehensive evaluation of glycoprotein changes should be examined and a battery of parameters would prove to be more informative. Therefore, the present study focused on multiple aspects of glycoprotein changes, which is an essential process in all cells. A critical assessment of the nature of this association can be of much importance in cancer diagnosis and management. It was thought worthwhile to study the significance of serum levels of: **(i)** alterations in glycoprotein constituents including sialic acid, fucose and seromucoid fraction (in terms of mucoid protein and hexoses), **(ii)** alterations in electrophoretic patterns of serum glycoproteins, **(iii)** changes in the levels of relevant enzymes including fucosidase, sialyl transferase and fucosyl transferase and **(iv)** alterations in glycosylation patterns in terms of fucosylation and sialylation using specific lectins, in patients with breast cancer and oral cavity cancer because these are the leading sites of cancer in Gujarat, India. Alterations of the markers in circulation were confirmed by analysing their levels in malignant tumour as well as normal tissues.

5.2 GLYCOPROTEIN CONSTITUENTS

Previous reports on changes in sialic acid, fucose and seromucoid fraction levels during malignancy have led to a wide belief that these glycoprotein

constituents may be essential for malignant cell differentiation. The elevated levels of sialic acid, fucose and seromucoid fractions are often found in blood through, increased turnover, secretion and or shedding from malignant cells (Alhadeff, 1989).

Increased levels of serum sialic acid have previously been measured in patients with malignancies of oral cavity (Sashikantha and Rao, 1994), ovary (Lagnana et al, 1998), colon and rectum (Bhuvarahamurthy et al, 1995; Feijoo et al, 1997). Verozin et al, (1990) have suggested that ratio of sialic acid to protein is a better marker than sialic acid alone and carcino embryonic antigen for detection of colorectal cancer. Serum levels of free, protein bound and total sialic acids as well as ratio of total sialic acid to total protein have been found to be elevated in other malignant diseases as well (Feijoo et al, 1997; Lagana et al, 1998; Paszkowaska et al, 1998; Romppanen et al, 1997). Elevated levels of sialic acid, fucose, hexoses and hexosamines are recently reported in cancer patients by Bhuvarahamurthy et al, (1995) and Arivazhagan et al, (1998). Sashikantha and Rao (1994) have reported raised fucose levels in sera of cancer patients as compared to healthy individuals. Fucose levels normalized by total proteins were significantly higher in cancer patients (Fernandez et al, 1997). Various attempts have been made to assess clinical utility of protein bound sialic acid, fucose and seromucoid fraction for detection and management of cancer (Gosh and Nayak, 1991; Waalkes et al, 1978). However, the numbers of subjects were smaller in previous reports. The precise relevance of these biomarkers with extent of disease, its impact on survival and response to therapy in patients with breast cancer and oral cavity cancer have not been systematically studied. Hence, it was necessary to focus on the glycoprotein changes in these malignancies. The results on glycoprotein constituents in present study were in accordance with the observations by various other workers. To the best of our knowledge, the present study validated data on the largest population of the patients with long-term post- treatment follow-ups which is an important feature of the work.

Serum glycoprotein constituents may be elevated in several other abnormal pathological conditions, like acute inflammation, high grade fever, rheumatoid arthritis, bacterial prostatis, benign breast diseases, pulmonal tuberculosis etc. (Listinsky et al, 1998; Romppanen et al, 1997). Previous reports on glycoprotein constituents in cancer have mostly compared the levels with healthy individuals. The goal of present study was to understand association of glycoprotein changes with malignancy. Therefore, the patients with BBD/OPC, served as pathological controls in the current study. We found that breast cancer as well as oral cavity cancer patients had significantly higher levels of different forms of sialic acid, fucose and seromucoid fraction as compared to the controls. The results showed significant rise in levels of all parameters except ratio of protein bound sialic acid to total protein in patients with breast cancer as compared to patients with BBD. Likewise, levels of the markers were significantly higher in untreated oral cavity cancer patients as compared to patients with OPC. In comparison between patients with OPC and healthy males, the former group showed significant rise in levels of the glycoprotein constituents except protein bound sialic acid and its ratio to total proteins, fucose and its ratio to total proteins as well as ratio of hexoses to total proteins. Present study also documented that elevations in serum glycoprotein constituents have significant positive correlation with stage of the malignant disease which is in accordance with reports of Baxi et al, (1991), Feijoo et al, (1997) and Gosh and Nayak (1991).

In order to assess the impact of serum concentrations of glycoprotein constituents on overall survival of patients, Kaplan Meier ~~product~~ was used. The patients were sub-grouped by their levels between biomarkers at the time of diagnosis. Statistically significant relationship between serum levels of the markers excluding free sialic acid and survival of breast cancer patients was observed. Similarly, the levels of protein bound sialic acid and its ratio to total protein, ratio of total sialic acid to total protein, fucose and its ratio to total protein as well as mucoid proteins and its ratio to total protein showed significant association with survival of oral cavity cancer patients. This is a poorly studied part of the previous work on glycoprotein constituents. Only a

few reports showing association of pretreatment levels of these markers with survival of the patients are available in the literature (Ogoshi et al, 1997). The current investigation also revealed significant prognostic value of glycoprotein constituents for cancer patients.

The value of a tumour marker in a given setting depends on two marker related characteristics; sensitivity and specificity. To determine the diagnostic values of present biomarkers, we have constructed ROC curves which account for both, specificity and sensitivity, simultaneously (Feinstein, 1985). As it can provide comparative assessment of markers, this analysis has gained increasing popularity in medical field in recent years. The ROC analysis provides an index of diagnostic accuracy i.e. independence of extra image decision factor and prior probabilities (Zweig and Compbell, 1993). Higher sensitivity and specificity of sialic acid, fucose and seromucoid fraction have been reported for various malignancies by Painbeni et al, (1997) and Baxi et al, (1991). In the current study also exhibited higher diagnostic accuracy of the biomarkers for breast cancer and oral cavity cancer.

Once the disease is diagnosed and treatment is started it is necessary to have evaluation parameters which can scant recurrent and/or metastatic disease at an early stage. Differences in therapeutic output are many a times difficult to explain only by clinical findings. Various investigators have reported significant correlation between continuously raised levels of sialic acid, fucose and seromucoid fraction and a worse prognosis in cancer patients (Bhuvarahamurthy et al, 1995; Fernandez et al, 1997; Painbeni et al, 1997; Sashikantha and Rao, 1994). However, the numbers of subjects in these reports were less. Our observations on a large population showed that the patients who did not respond to therapy had higher levels of biomarkers than the patients who showed complete and sustained biochemical response to therapy.

As described by various investigators, major possibilities for the increase in glycoprotein associated carbohydrates are: **(i)** increased carbohydrate

contents of normal proteins as a result of cell damage, **(ii)** glycoprotein production by tumour itself, which are otherwise absent or found in lower concentartions and **(iii)** increased synthesis of glycoproteins by liver and/or lymphoreticular tissue as an acute phase response to the disease conditions. The appearance of different glycoforms of proteins appears to be a sensitive reflection of the physiological and biochemical conditions present in the tissue source at the time of synthesis and release (Rademacher et al, 1988). To examine the origin of altered serological concentrations of biomarkers, present study has also included malignant and adjacent normal tissues for analysis of glycoprotein constituents. The study revealed significantly higher concentrations of sialic acid, fucose and seromucoid fraction in tissue homogenates of malignant tumours as compared to that of surrounding normal tissues. Significantly higher levels of sialic acid in tumour tissue have also been reported by various workers (Feijoo et al, 1997; Suer et al, 1996). Similar observations for abnormal levels of these glycoprotein constituents have been documented in tissue extracts of endometrium, lung, breast, cervix and liver cancer (Wang et al, 1995; Fernandez et al, 1997).

However, previous reports have not correlated presence of circulating markers with their concentrations in tumour tissues. The results of the present study exhibited that the alterations of glycoprotein constituents in serum are a reflection of the alterations present in the tumour tissues. Thus, the present study addressed the role of serum levels of sialic acid, fucose and seromucoid fraction in providing prognostic information and its potentials to serve as a guide for therapeutic monitoring.

5.3 CHANGES IN GLYCOPROTEIN ELECTROPHORETIC PATTERNS

Variations in serum protein values have provided clinically useful informations in various diseases. Since the first report of tumour marker, Bences Jones protein, serum proteins have drawn considerable interest. Changes in serum glycoprotein levels are characteristics of many pathological conditions

pathological conditions including malignancy (Shetlar, 1961). Most of the tumour markers known so far are also glycoproteins in nature. The glycosylated proteins including α-fetoprotein, CEA, CA-125, CD44 and CA 19-9 are helpful to detect presence and recurrence of various malignancies (Hugland et al, 1997; Martin et al, 1997; Nakata et al, 1998). Glycoproteins like fibronectin, laminin, epiglycanin and mammary tumour glycoprotein (Hugland et al, 1997) have been distinctly associated with malignancy. Serum levels of TAG-72 (Tumor associated glycoprotein) are reported to be elevated in cancer patients (Gonzalez et al, 1996). TAG-72 levels were found to have a good correlation with clinical course of the disease during follow-up in lung cancer patients (Scambia et al, 1990). Elevations in serum levels of acute phase proteins including: α-1 antitrypsin, haptoglobin, β-2 microglobulin and decline in albumin have been reported to be useful diagnostic and prognostic indicators (Suttar et al, 1997; Suaraz-Nieto et al, 1986; Thomson and Turner, 1987; Ogoshi et al, 1997). Over expression of 170 k Da transmembrane glycoprotein have been reported in malignant solid tumours (Chuman et al, 1996; Filipitis, 1997). S-100 protein levels have been found to show good correlation with both, time of recurrence and survival of cancer patients (Miliotes et al, 1996). Some reports point towards a significant correlation between levels of circulating immune complexes, tumour burden and prognosis (Balaraman et al, 1987; Baselar et al, 1987; Chester et al, 1990).

Earlier reports have elucidated relationship of protein electrophoretic patterns with malignancy (Adan and Farber, 1982; Chorvath et al, 1983; Salasvov et al, 1980). Putnam and Udin (1953) and Singh et al, (1984) observed similar finding suggesting neoplastic proliferation of a single clone of plasma cells engaged in the production of monoclonal proteins, i.e. immunoglobulins. Significantly elevated levels of total protein and globulins as well as lower levels of albumin were observed in patients with multiple myeloma (Chen and Magalhaes, 1990). Earlier studies have documented decreased concentrations of albumin and increased values of globulin levels in cancer patients (Altara, 1993; Maisin et al, 1972; Salasvov et al, 1980).

Electrophoretic analysis of several enzymes which are glycoproteins in nature revealed significant clinical information in cancer patients (Harmenberg et al, 1989; Nigam et al, 1996; Patel et al, 1994).

Sialic acid, fucose and seromucoid fraction are the basic constituents in the structure of all glycoproteins. Therefore, any change in glycoprotein will account for the changes in sialic acid, fucose as well as seromucoid fraction levels and vice versa. Serum glycoprotein electrophoresis which separates the major circulating proteins can be highly specific for preneoplastic and malignant conditions. Considering the fact that electrophoretic analysis can provide a comprehensive view on multiple proteins, we have analysed electrophoretic patterns of serum glycoproteins. Our preliminary reports (Patel et al, 1995, Patel et al, 1997) on these significant observations received considerable attention and stimulated a great interest among other workers. Therefore, the present study focussed on alterations in circulating glycoprotein levels in patients with BBD/OPC and cancer patients. The purpose was to investigate whether the alterations in glycoprotein constituents are reflected by the changes in glycoprotein electrophoretic patterns or not? The periodic acid schiff's(PAS) staining of protein electrophoresis revealed sharp and multiple bands in gamma region which are not that clear when stained with CBB for total proteins. The results revealed decreased albumin region glycoproteins and elevated gamma region glycoproteins in cancer patients which supports other reports.

Number of glycoprotein bands in gamma region was highest in cancer patients followed by patients with BBD/OPC which was followed by controls. The current results revealed more number of glycoprotein bands in sera of cancer patients as compared to the patients with BBD/OPC as well as the controls. Noteworthy observation of the present study is the more frequent presence of an extra glycoprotein band between beta and gamma region among cancer patients. The presence of more number of glycoprotein bands in cancer patients may be due to altered glycosylation of the proteins which are present in the healthy individuals. The elevations in gamma region

glycoproteins may be due to the presence of circulating immune complexes in sera of cancer patients which has been reported earlier (Balaram et al, 1987; Baselar et al, 1987; Chester et al, 1990). Another reason for elevations in gamma region glycoproteins may be due to the presence of high molecular weight glycoproteins reported in cancer patients (Aziz et al, 1988; Gupta et al, 1980). The presence of more number of glycoprotein bands in sera of cancer patients may be due to the presence of tumour associated antigens. The tumour associated antigens have been an attractive target for immunotherapy of cancer. The results also correlate with the report of O'Brien et al, (1991) who found more frequent presence of five glycoproteins having molecular weight 38, 46, 82, 95 and 178 K Da. Silverstri et al (1998) also reported that 90 K Da glycoprotein belonging to the scavenger receptor family is elevated in cancer patients. The resistance of cancer cells to therapeutic action of anticancer treatment is a serious clinical problem which is often encountered during management of cancer patients. Few of the glycoproteins like P-glycoprotein seem to be responsible for resistance of drug (Ng I.Ø et al, 1998). The present study showed unchanged glycoprotein electrophoretic patterns which were associated with unfavourable treatment outcome. The persistent presence of all gamma region glycoprotein bands may correlate with drug resistance markers. The present data indicates that favourable treatment outcome was associated with disappearance of various glycoprotein bands in gamma region and elevations in albumin region glycoproteins. The current results indicated that alterations in glycoprotein electrophoretic patterns were strong indicators of disease status in cancer patients receiving therapy. The literature survey revealed that these observations on electrophoretic changes in glycoproteins are not reported previously. Actual cause for alterations in serum glycoproteins during malignancy is not understood properly. Glycoproteins seem to play an undefined role in cancer. Glycoprotein bands, which were associated with presence of the disease, may be due to the cancer associated antigens, drug resistant proteins or circulating immune complexes, which are reported earlier by various workers. The results suggest that assessment of glycoprotein patterns can furnish the decision making step related to the

treatment of cancer with an additional and powerful tool. Thus, it can also minimise or even omit estimations of additional tumour markers using radioimmuno assays and molecular biology methods. The detection of glycoprotein alterations by electrophoresis as evidenced by the current report may provide a simple means for diagnosis, prognostication and treatment monitoring of patients with the malignant solid tumours.

5.4 SIALOPROTEINS AND FUCOPROTEINS

Majorities of the serum proteins are glycosylated. When disease is present, subtle changes occur in these glycosylation. The knowledge of the way in which serum proteins are glycosylated in cancer patients will contribute to the understanding of glycoprotein functions. It can be used as a basis for understanding of cancer biology. These changes could provide the basis for clinically useful informations in various diseases. It is reported that the glycosylation of proteins play an important role in the functions that they perform during malignant transformations (Feizi and Childs, 1987; Paulson and Colley, 1989; Redmacher et al, 1988). Some characteristic features of tumour cells, such as the ability to escape from immune system recognition have been related to the presence of altered oligosaccharide sequences on their surface (Bloscher et al, 1989; Bresalier et al, 1990; Bruyneel et al, 1990; Collard et al., 1986; Dennis, 1992; Pettijon et al, 1988). Employing *in vitro* and *in vivo* approaches, numerous investigators have found differences in glycosylation patterns accompanying malignant transformation in animals and humans. The carbohydrate moieties of glycoconjugates may influence growth and cell-cell interaction and thus may be important in development of malignancy (Hakomori, 1989). The Glycosylation changes are independent of protein synthesis (Van Dijk et al, 1994). Previous results are centered largely around the demonstration of increased levels of carbohydrates of the carbohydrate-protein complexes in sera of cancer patients. Terminal glycosylation processing led to the branching and heterogeneity characteristic of many glycoprotein glycans (Schachter, 1986). Increased branching is the

feature of a number glycoproteins derived from cancerous tissue. The major carbohydrate components of mammalian glycoproteins are D-galactose, D-mannose, sialic acid and L-fucose (Nigam and Contero, 1973). Among these, sialic acid and fucose being terminal sugars, most studies, like the present study have focussed on sialylation and fucosylation of proteins. The data from our laboratory and others indicate elevations in protein bound sialic acid and fucose levels in various malignancies (Sashikantha and Rao, 1994; Shahangian et al, 1991; Thompson et al, 1992; Turner et al, 1985). The elevations in fucose and sialic acid contents cannot be explained only by the production of new proteins because only a few new glycoproteins are seen on electrophotesis. The changes found in sialic acid and fucose between normal and malignant conditions could be due to an overall higher amount of sialic acid and fucose. A selective increase in existing specific sialylated and fucosylated sequence or a tumour associated *de novo* synthesis of specific sialylated sequence may also be anticipated.

The pathologic variations in different glycoforms of glycoproteins in serum most likely result from changes in glycosylation process during their biosynthesis. Variations in the structure of an oligosaccharide glycan have been previously referred to as microheterogeneity. Minor microheterogeneity can be caused by variations in sialic acid, galactose and/or fucose contents (Thompson et al, 1988; Thompson and Turner, 1987). Specific alterations in the glycosylation of acute phase proteins occurs in many patho-physiological states (Turner, 1992). The changes in serum sialoglycoproteins that profile malignancy are shared by other disease status, but correlation of malignant cells with increased or abnormal sialoproteins is found to be different (Alhadeff, 1989; Alhadeff and Holzinger 1982; Bolmer and Davidson, 1981; Feizi and Childs, 1987). It was reported by Muryama et al (1997) that alpha 2-6 sialylation recognised by Sambucus nigra is different than sialylated Tn antigens. Sialic acid and S-100 proteins are found to be useful for prediction of recurrence and survival of patients and could be useful in the clinical detection of patients with malignancy (Miliotes et al, 1996). Bellahcence (1996) reported that bone-sialoprotein (70-80 k Da) in

primary human breast cancer was associated with poor survival. Increased sialylation especially involving sialyl Lewis A and sialy Lewis X determinants has been reported in breast cancer. Elevations of sialylation has been reported to be associated with poor prognosis and resistance to cancer therapy (Vierbuchen et al 1995).

Altered fucosylation of various proteins has also been reported to be essential for various functions of cell during pathological conditions (Glick, 1978). Fucosylation of certain tumour associated antigens like, Haptoglobin α-feto-protein and CEA are also reported in cancer patients (Turner et al, 1995; Aoyagi et al, 1993). Kondo et al. (1994) reported fucosylated IgG in sera of cancer patients. Macbeth and Bekesi (1962) have suggested that fucose of neutral glycoproteins may be elevated exclusively in malignant diseases, even including clinically localised carcinoma of breast. Specific sets of fucosylated glycans appear in certain mammalian tissues and cells at defined period of development (Feizi and Childs, 1987; Pettigon et al, 1988; Santer and Glick, 1983). Various reports have suggested alterations in protein bound fucose levels in sera of cancer patients which showed correlation with clinical status of the patients during follow-up. The present study also found similar results. It has been suggested that increased Lewis antigen expression results from increased fucose transfer (Inoue et al, 1990). Haptoglobin is normally synthesised by the liver (Koj, 1974) but it may also be synthesised by some tumour cells (Yoshimura et al, 1978). Thompson et al, (1992) have reported higher expression of fucosylated haptoglobin in sera of cancer patients which was in accordance to tumour burden. The authors also reported that fucosylated haptoglobin in cancer sera showed correlation with fucose levels. The SL_eX which is having terminal fucose residue has been found on the surface of various human cancer cells (Fukushima et al, 1984; Majuri et al, 1994). Its expression plays an important role in E-selectin-mediated adhesion to activated endothelium (Philips et al, 1990; Walz et al, 1990; Tiemeyer et al, 1991). The subsets of fucosylated cell surface carbohydrate molecules serve as an oligosaccharide ligands for two members of the selectin family of cell adhesion receptors. Increased

sialylation and fucosylation of acute-phase proteins was reported in cancer patients sera by Matei (1997). Internally fucosylated and sialylated glycan may also function in cell surface adhesive interactions (Geolż et al, 1990; Macher et al, 1991). Much evidence has accumulated indicating that sialylated and/or fucosylated lactosamino glycans are involved in fetal development, tumorigenesis, hematopoietic cell differentiation and leukocyte trafficking (Fukuda, 1994).

High sugar specificity of lectins makes them an ideal tool for identifying structural features of the oligosacharide moieties of glycoproteins and changes there of. The availability of an increasing number of lectins which are proteins that specifically bind to defined carbohydrate structure (Grant and Peters, 1984; Lis and Sharon, 1986), ~~This knowledge~~ has contributed significantly to study of malignant cell glycoconjugates in the last decade. (Vierbuchen et al, 1995; Schumacher, 1996)Sambucus Nigra, a sialic acid specific lectin selectively binds to terminal sialic acid residues which are linked via α-2-6 linkage during malignancy, has been reported by various workers (Sata et al, 1991; Muryama et al, 1997). Lectin Lotus Tetragonolobus has affinity for α-L fucosyl residue and it has unusual high affinity for α-L fucose residues of oligosaccharide chain (Periere and Kabat, 1974). Lotus specificity is directed towards fucose that is linked in either α (1,2) position to a sub-terminal galactose or in a α (1,6) position to a N-N' di-acetylchitobiose core (Petryniak and Goldstein, 1986). Whether the sialylation and fucosylation of serum proteins are altered in cancer or not is an unclear objective. Therefore, in the present study serum sialoproteins and fucoproteins were isolated using specific lectins.

The current investigation found more number of sialoprotein bands among cancer patients as compared to controls as well as patients with BBD/OPC. Also, expression of some of the fucoproteins was higher in cancer patients. These findings suggested that the presence of the additional bands may also be due to sialylated tumour associated antigens. We have observed more number of proteins having terminal L-fucose residues in patients with

malignant solid tumours as compared to healthy individuals and patients with BBD/OPC. The current findings on glycoprotein constituents (sialic acid and fucose) revealed significant use in prognostication and treatment monitoring which go hand in hand with the increased sialylation and fucosylation observed in terms of elevated sialoproteins and fucoproteins. Alterations in cellular glycosylation patterns is a routinely assessed feature in diverse type of neoplastic diseases. Such changes may occur as: **(i)** incomplete form of carbohydrate structure, **(ii)** re-expression of oncofetal antigens that normally exist during the embryonic development but either disappeared or become minimally expressed in the corresponding adult tissue or **(iii)** organizational changes due to demasking or increased expression of normally expressed carbohydrate structure. Other possible explanations include increased production of pre-existing serum glycoproteins and/or alterations in the sugar moieties of these molecules.

5.5 ALTERATIONS IN THE ENZYMES

Protein glycosylation has been demonstrated to play a critical role during malignant transformation due to their vital importance in number of biological processes (Kobata and Takasaki 1993, Varki 1993). The expression of glycosyltransferases play an important role in determining glycosylation patterns. (Kornfield and Kornfield, 1980; Paulson, 1989). Sialic acid and fucose have been particularly associated with the modulation of cell adhesion (Kelm and Schauer 1997, Glick 1978). Therefore the glycosylation changes in cancer mainly include sialylation and fucosylation (Turner, 1992; van Dijk et al, 1994). Subtle changes occur in the process of glycosylation through a series of enzymatic steps during post-translational modifications. Glycosidase and glycosyl transferases that respectively catalyse the stepwise trimming and addition of sugar residue are generally considered as working in a co-ordinated and highly ordered fashion to form N-glycans. On the basis of this assembly line concept using N-linked glycan structure as a milestone marking of the malignancy currently makes fast progress. The mechanism, which

regulates the expression of glycosyl transferase during ontogenic development, cell differentiation and neoplastic transformation is not completely understood.

The transfer of sialic acid and fucose from nucleotide sugar to N-linked sugar chain is found to occur as the final step of oligosaccharide biosynthesis. Therefore, the enzymes involved in addition and deletion of terminal sugars were analysed for better understanding of the mechanism of elevations in sialic acid and fucose values during malignancy. To assess the control of biosynthesis of glycoconjugates, we have studied serum sialyl transferase and fucosyl transferase activities. Due to the association of malignant transformation with the degradation of terminal sialyl or fucosyl residue of glycoproteins, the study included analysis of sialidase and fucosidase levels from sera of cancer patients. Earlier, Rothenberg et al, (1996) in their preliminary study found declined levels of neuraminidase in patients with breast cancer as compared to healthy donors. They postulated that high sialic acid levels when associated with inadequate neuraminidase activity may predispose to an increased risk of cancer. Miyagi et al. (1994) have also reported decreased sialidase activity in transformed cells. However, Rotherburg et al, (1994) in their study observed significantly higher activity of sialidase in tumour tissues as compared to normal mucosa. The present study, attempted to analyse serum sialidase activities by flourimetric assay (Potier et al,1979) to elicit its relationship with malignancy. Due to very low concentration of sialidase in serum we were unable to analyse the activity of sialidase. We have not come across any other study also of sialidase activity in human serum.

Takahasi et al (1994) have reported that serum levels of L-fucosidase can be an useful marker in the diagnosis of hepatocellular cancer. However, the precise mechanism behind the elevations in fucosidase levels have not been determined. Hutchinson et al, (1991) and Abdel et al, (1996) have reported elevated fucosidase activities in sera of cancer patients. Barlow et al, in 1981 has reported elevations in serum fucosidase concentrations among patients with epithelial cancer. Bhuvarahamurthy et al, (1995) have reported

significantly higher activity of fucosidase in cancer patients which was normalized after radiotherapy. The current findings observed declined fucosidase levels in patients with breast cancer and oral cavity cancer as compared to patients with BBD/OPC. The fucosidase levels also showed correlation with stage of the disease. Variations in the enzyme levels were also found to be helpful in predicting disease free survival in cancer patients. To assess therapeutic response, the enzyme levels were compared between pre and post-therapeutic blood samples. The fucosidase levels showed significant correlations with effectiveness of therapy in cancer patients. Various other investigators have also reported serum fucosidase levels to be a valuable tool in evaluating therapeutic response in patients with cancer. The results of current study emphasise clinical value of serum fucosidase for cancer patients at diagnosis and during follow-up. The precise mechanisms behind the elevations of this parameter have not been determined, however; various hypothesis are put forward for possible causes of alterations of the enzyme levels. The cancer cells are believed to produce extracellular matrix degrading enzymes, various protease as well as exo and endo glycosidases. The secretions of endoglycosidase from cancerous cells into peripheral blood can result in deglycosylation of some protein in plasma (Yamamoto et al, 1995). Wiederschain et al, (1971) have reported presence of α fucosidase in human placenta, amniotic fluid and fetus liver during gestation.

Gessner et al, (1993) demonstrated that determination of α-2-6 sialyl transferase in colorectal tumour tissue and sera of cancer patients may be a new means for tumour detection and monitoring. Higher expression of sialyl transferase was also reported in 79% of malignant brain tumours and the enzyme was undetectable in normal brain tissues (Yamamoto et al, 1995). Shimada et al, (1995) have reported that increased excretion of glycosidically bound sialic acid in urine of cancer patients reflects elevations of sialyl transferase ~~sialyl transferase~~ activity in tumour tissue. Increased serum sialyl transferase activities have been reported in patients with gastrointestinal cancer as compared with healthy individuals (Ganzinger and Deutsch 1980).

Ronquist and Nou (1983) have reported elevated levels of sialyl transferase in patients with lung cancer as compared to patients with benign pulmonary diseases. Increased plasma sialyl transferase has also been observed in patients with breast cancer (Linang et al, 1993), colon cancer (Griffiths and Reynold, 1982), brain tumour (Yamamoto et al, 1995), and variety of other cancers (Altara 1993; Baker et al 1987; Schawartz et al, 1984). Present study revealed higher activities of sialyl transferase in sera of cancer patients as compared to healthy individuals as well as pathological controls. The study also evaluated serum enzyme levels during follow-up to find out their value in treatment monitoring. A significant decline in the levels of sialyl transferase was associated with favourable treatment response. Serum sialyl transferase levels significantly correlated with disease status during follow-up in breast cancer as well as oral cavity cancer patients. As reported by Dao et al. (1980) as well as De and Hardy (1990) serum sialyl transferase is an important biomarker for assessing response to anticancer therapy. The changes in serum levels of certain glycoprotein tumour markers like CEA, CA19-9 along with sialyl transferase after chemotherapy are documented to be ~~an~~ excellent prognostic indicators for patients with cancer (Nakata et al, 1998).

Significantly increased plasma fucosyl transferase values in patients with liver carcinoma than other liver disorders were reported by Hada (1997). Fucosyl transferase were reported as useful tumour marker (Amano et al,1992). Increased values of fucosyl transferase have been reported in the sera of patients with various malignancies (Chandrasekaran et al, 1992; Hutchinson 1991; Ronquist and Nou 1983;Thompson et al, 1992;, Yazawa et al 1989). In a former study, protein bound serum fucose levels were found to be elevated in various malignancies and were correlated with the increase in fucosyl transferase activity in all the malignancies studied excluding cervical cancer patients (Sen et al, 1983). Higher levels of fucosyl transferase have been reported in patients with colon and mammary carcinoma by Bauer et al (1978). Yazawa et al, (1988) have found cancer associated elevations in fucosyl transferase activity in human serum,

however; patients with non-neoplastic diseases showed normal fucosyl transferase levels.

The present study found significantly higher levels of fucosyltransferase among the untreated cancer patients as compared to healthy individuals and patients with OPC/BBD. Serial estimations of fucosyl transferase were carried out to evaluate its importance in assessing response to anticancer therapy. The nonresponders showed elevated or similar fucosyl transferase levels as compared to their levels at the time of diagnosis. While, patients without demonstrable malignant disease exhibited lower levels of fucosyl transferase as compared to untreated cancer patients. Serum fucosyl transferase levels can be considered as additional tool for evaluation of malignancy and effectiveness of treatment.

Yazawa et al, (1989) has suggested that changes in fucosyl transferase levels are useful in predicting treatment outcome in cancer patients. In patients with breast, colon or gastric cancer, removal of tumour was associated with significant reduction in serum activity of fucosyl transferase (Asao et al, 1989). Bauer et al in 1978 have reported that decrease in human serum fucosyl transferase is an indicator of successful tumour therapy. They have found a decrease in fucose incorporation in its endogenous acceptor after 4 to 6 days of surgery among the patients with colon and breast cancer. Increased sialyl transferase and fucosyl transferase activities may be responsible for increased expression of cell surface glycoconjugates. The current results supported the notion that the alterations seen in cell surface glycoconjugates during oncogenic transformation can be the result of altered expression of glycosyl transferases. Sialyl transferase and fucosyl transferase which respectively catalyse terminal addition of sialic acid and fucose for maturing carbohydrate chains of asialo and afuco glycans are believed to be elevated in cancer patients. The role of fucosyl transferase in the accumulation of fucoglycolipids in malignant tissues has been investigated in humans (Holmes et al, 1986). The relationship between the mechanism of apoptosis (in tumour tissues) and sialic acid on the termini of sugar chains of

glycoconjugates has been studied. The results have 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 presence of sialyl transferase and fucosyl transferase in malignant cells could lead to altered or even unique glycoconjugates. Possible mechanisms for elevations in enzyme activities were proposed by various workers. Glycosyl transferases are reported to be either secreted by neoplastic cells (Reutter and Bauer 1978) or released during cellular degradation (Kim et al, 1982). One of the mechanisms for appearance of these enzymes in serum may be by the shedding of plasma membrane constituents into systemic circulation of host (Chatterji et al 1976, 1979). Alternatively, it may be due to the over production or release from the lysed tumour cells.

The evaluations of serum fucosidase levels revealed its significant clinical value for cancer patients. Enzymes associated with glycan moieties are thought to be either secreted by neoplastic cells (Reutter and Bauer, 1978) or may be released during cellular degradation (Kim et al, 1982). Another mechanism for the appearance of these enzymes in serum may be by the shedding of plasma membrane constituents into systemic circulation of host (Chatterji et al, 1979). Various studies have shown significantly higher levels of fucosidase in tumour tissues (Wang et al, 1995; Gil-Martin et al, 1996; Hutchinson et al, 1991). Significant elevations in fucose levels have been observed in malignant tumour tissues obtained from patients with various malignancies (Hutchinson et al, 1991; Mas et al, 1998; Wang et al, 1995). An increase in the sialyl transferase activity of malignant tumours have been documented (Akamatsu et al, 1996). However, the association between circulating levels of these enzymes and their concentrations in tumour tissues were not studied by previous workers. To study the correlation of circulating enzyme levels with that in tumour tissues, the present investigation analysed all the three enzymes in malignant tumours and adjacent normal tissues. As the sialidase activity was not detectable in serum, it was not estimated in tumour tissues. Activities of all the three enzymes showed statistically

significant elevations in tumour tissues as compared to the normal tissues. Elevations in the enzyme levels of tumour tissues are in accordance to their circulatory levels.

AS AN OVERALL VIEW ON THE DATA, the current investigation found significantly higher levels of glycoprotein constituents as compared to healthy individuals. The levels of glycoprotein constituents and fucosidase showed positive correlation with stage-wise disease activity. Higher levels of sugar residues were associated with poor prognosis. The patients with poor prognosis showed elevated levels of the markers throughout the follow-up duration. Further, markers were raised before clinical evidence of recurrent metastatic disease. The alterations in sialic acid and fucose levels also correlated with extent of sialylation and fucosylation as well as the enzymes responsible for the same. The elevations in carbohydrate moieties in cancer patients' sera were accompanied by elevations in circulating glycoproteins. Altered glycoprotein levels correlated well with alterations in the glycosylation changes. The present study used radio assay for estimation of sialyl transferase and fucosyl transferase. Since the results on sialyl transferase and fucosyl transferase are also interesting, ELISA assays using lectins should be standardised for routine assays. The results implied that measurement of sialic acid, fucose, mucoid protein, hexoses, fucosidase, sialyl transferase, fucosyl transferase and glycoprotein electrograms are of clinical value in monitoring clinical course of cancer patients as well as in assisting the diagnosis of breast and oral cavity cancer. The analysis of present tumour markers can be additional tools for diagnosis, prognostication and treatment monitoring of cancer patients.