Discussion

"The best way to have an idea is to have lots of good ideas".

- Linus Pauling

5: DISCUSSION

5.1: SIGNIFICANCE AND APPROACH OF THE PRESENT STUDY

Oral cancer is a major global threat causing great morbidity and mortality that have not improved in decades. It is currently the most frequent cause of cancer-related deaths in India. An array of unresolved questions exists in the fight against cancer. Therefore, more intense efforts are required against this life threatening disease. The late presentation of majority of the oral cancer cases reduces the overall survival. In the present study, around 72% of the oral cancer patients were having advanced disease (stage III + IV) (**Table-3.3**). In such cases, the death rate is very high due to loco regional failure and/or lymphnode metastasis even after successful surgery. As with any other cancer, treatment of oral cancer is best if administered at the earliest stage. This maximizes chances of successful treatment. Hence, multiple approaches are needed to reduce the devastating consequences of the disease. Although oral cancer undoubtedly has a multifaceted etiology, tobacco use is widely considered to be its major risk factor either in the form of smokeless tobacco chewing or smoking (Gupta and Warnakulsauriya, 2002). Majority of the patients with oral cancer (97%) and patients with OPC (98%) enrolled in this study were chronic tobacco habitués (**Table-3.2**).

Oral cancer development is a complicated process associated with multiple pathological changes in oral tissues. Mainly two hypotheses form the basis for the oral carcinogenesis: (i) Multi-step carcinogenesis and (ii) Field cancerization. Oral cancer most often preceded by specific oral lesions leukoplakia and oral submucous fibrosis that are called precancers. Hence, it is important to know the molecular and biochemical changes taking place at each steps of carcinogenesis. Various researchers have compared alterations in different biochemical changes between healthy individuals and oral cancer patients. However, it is essential to study markers in patients with OPC who are at higher risk to develop oral cancer. Therefore, the current retrospective study included healthy individuals as controls, patients with OPC and untreated oral cancer patients, which may demonstrate the molecular insights

into oral carcinogenesis. There is growing number of molecular markers for oral squamous cell carcinoma; nevertheless prognostic relevance of the markers is not clearly indicated. Therefore, the present study also included follow-ups of oral cancer patients during and/or after anticancer treatment (**Table-3.3**), which successfully demonstrated their usefulness in prognostication and treatment monitoring of oral cancer.

Malignant transformation of a cell involves numerous molecular and biochemical alterations at transcriptional, translational and post-translational levels. Glycosylation is one of the key post-translational modifications that can influence both secondary structure and functions of the several protein backbones. Most of the membrane bound and secretary proteins are glycoproteins. The structural uniqueness and micro heterogeneity of glycoproteins has fascinated many scientists. A profound derangement of cell glycosylation is a prominent effect of neoplastic transformation (Dall'Olio, 1996). Changes in protein glycosylation pattern are responsible for modification of various important cellular functions as well as protein stability and activity. Transformed tumour cells escape from normal immune surveillance by displaying glycosylated structures on their surface. Under normal circumstances cells are held together with cell adhesion molecules and allow interactions between numerous proteins on the cell surface. Hence, difference in metastatic properties of various tumours might be related to differences in their cell surface components. Glycosylation process occurs in the endoplasmic reticulum and golgi compartments of the cell, which involves complex series of reactions catalyzed by various membrane-bound glycosyltransferases (Dell and Morris, 2001). Biosynthesis of complex-type structures is completed by a variety of "capping" reactions; the most important is the addition of terminal sugars sialic acid and fucose. Although the biosynthesis and functions of sialylated and fucosylated sequences are beginning to be understood, the biochemical properties, expression and regulation of these important enzymes remains to be explored. Terminal glycosylation has been a recurring theme of the research. The present study

investigated the biochemical changes associated with the chain events of terminal sialylation and fucosylation, which are believed to be one of the most pathologically relevant glycosylation changes in oral carcinogenesis. Glycosylation of the membrane-bound or secretary proteins have been referred as "chemical passport for the exit through the cellular membrane" (Eylar, 1965). These glycoproteins can be released into the circulation through increased turnover, secretion and/or shedding from malignant cells and are of considerable interest for their potential diagnostic and prognostic value. The changes in serum glycoprotein levels are characteristic of many pathological conditions including cancer.

The present study focused on the role of terminal glycoprotein alterations in tissue and serum of oral carcinoma patients mainly for three reasons: (i) to evaluate the biochemical changes taking place during oral carcinogenesis (ii) to understand the systemic effects of tumours on the host and (iii) to assess role in early cancer diagnosis and prognostication. To evaluate the basis of altered serological concentrations of biomarkers, tissue samples were analysed. Determination of biochemical markers in primary tumour and adjacent normal mucosa may have prognostic significance in the patients. Activation of these biochemical markers in adjacent normal mucosa could point towards an aggressive tumour phenotype. This means that, the presence of these molecules in adjacent normal tissues may have beneficiary effects on tumour growth and thereby worst prognosis. Serum levels of the markers may reflect the tissue damage, tissue hypertrophy and neoplastic transformation and hence play an important role in the management of patients as a less invasive procedure. Hence, the markers in tissue as well as serum samples were analyzed, so that they can be used as surrogate markers of tissue levels in oral cancer. Also, the idea of screening and following patients with oral cancer by serum tests is appealing because of possibility of repeated sampling and less-invasiveness.

5.2: ALTERATIONS IN CIRCULATING AND TISSUE LEVELS OF TOTAL SIALIC ACID, SIALOPROTEINS, SIALYLTRANSFERASES AND SIALIDASE

Several studies on serum or tissue levels of total sialic acid, sialoproteins, sialyltransferases and sialidase in cancer patients have been published. However, so far, studies in which all the parameters were simultaneously evaluated in serum and tissue from the patients, especially in oral cancer have not been reported.

In the present investigation, serum analysis (**Figure-4.2A & B**) revealed that TSA and TSA/TP levels were significantly elevated in untreated oral cancer patients and patients with OPC than healthy individuals. Further, TSA and TSA/TP levels were increased in patients with OPC as compared to healthy individuals. It has been reported that neoplastic transformation associated with elevations in serum TSA. These observations match with previous reports by Rao et al. (1998) and from our laboratory (Raval et al., 2003), which demonstrated alterations in different forms of serum sialic acid in patients with oral cancer and OPC. This suggested that malignant transformation brings changes in cell surface glycoconjugates. The significant elevations in TSA/TP in patients suggested that sialic acid changes are primary phenomena during malignancy rather than the secondary effect of the protein changes. Plucinsky et al. (1986) and Feijoo et al. (1997) also found TSA/TP as a better marker than TSA. The significant elevation of sialic acid in patients with OPC could reflect the early changes during malignant transformation of the cell. Thus, circulating levels of sialic acid appeared to reflect tumour burden and correlated well with the stage of primary lesion and presence of distant metastasis. Much attention has been devoted to study serum sialic acid in cancer patients with the aim of evaluating its role as a tumour marker, and its possible implication in the metastatic spread of malignancy. However, the origin of increased serum sialic acid levels remains unclear. Several possibilities are being considered which include: (i) an intensified release of sialic acid-rich glycoconjugates from tumour cells, (ii) an increased

concentration and/or glycosylation of normal serum glycoproteins, or (iii) an output of acute phase proteins from the liver as a result of unspecified secondary inflammatory reaction (Narayanan, 1994). Tissue analysis in the present study showed significant higher TSA levels in malignant tissues as compared with their adjacent normal tissues (**Figure-4.13, Table-4.6**), which implies the hypersialylation of tumour cell surface glycoproteins. Previous studies have found increased serum TSA in various malignancies (Aranganathan et al., 2005; Rajpura et al., 2005; Raval et al., 2003; Rao et al., 1998; Patel et al., 1995; Narayanan, 1994), which reflected its diagnostic and prognostic value. Paszkowska et al. (1998) and Feijoo et al. (1997) are among the few investigators who studied tissue and serum TSA in colon cancer and endometrial cancer respectively. However, to the best of our knowledge, this is the only study till date, which evaluated TSA levels simultaneously from serum and tissues in oral cancer patients.

In this study, grade III poorly differentiated tumours showed significantly higher levels of serum TSA and TSA/TP (**Figure-4.9**). Tissue or serum sialic acid levels did not show any significant correlations with other histological characteristics of tumour (**Table-4.4**). Increased serum and tissue levels of sialic acid have been correlated well with disease progression (Raval et al., 2003; Paszkowska et al., 1998; Feijoo et al., 1997), tumour differentiation and grade (Berbec et al., 1999) in different malignancies. Thus, more knowledge about the regulation and the sub-cellular site of sialic acid metabolism is required, not only for better indulgent of the biological functions, but to understand synthesis and turnover of sialic acid and that could be correlated with pathological processes like tumour growth.

The increased levels of sialic acid could lead to altered or even unique sialoglycoproteins with different linkages of sialic acid in malignant cells. Several investigators have suggested the linkage of sialic acid in development of an invasive phenotype in cancer. The present study detected tissues and serum levels of α2,6- and α2,3-sialoproteins using biotin-conjugated lectins

SNA and MAL respectively. There are no previous reports on changes in α2,6- and α2,3-sialoproteins in patients with oral cancer as well as OPC. The current line of evidence suggest that most of the lactosamine antennae are substituted with sialic acid with increased β1,6- branching may contribute to the carcinoma-associated increase in sialic acid thereby increased sialylation. It is generally thought that the oligosaccharide structure is complex and regulated mainly by the activity of the cognate glycosyltransferase. The use of specific lectins can be a useful approach to analyze the complex oligosaccharide sequences of various glycoproteins. During past years, lectins that discriminate between various types of sialylated sequence have been reported (Knibbs et al., 1991; Shibuya et al., 1987).

Present work demonstrated significant high levels of α2,6-sialoproteins and α2,3-sialoproteins in malignant tissues as compared with their adjacent normal tissues (**Table-4.6; Figure-4.14A & C, 4.15A & C**). Moreover, high α2,6- and α2,3-sialoproteins were observed in poorly differentiated malignant tissues than those in well and moderately differentiated tumours (**Figure-4.19**). α2,6-sialoproteins and α2,3-sialoprotein expressions in OPC tissues were also higher than their adjacent normal tissues, but the difference was not significant (**Table-4.7; Figure-4.14B, 4.15B**). Sata et al. (1991) have demonstrated histochemical detection of sialoproteins using both linkage specific lectins in colon carcinoma and dysplasia. They observed that epithelial normal colon mucosa and mild dysplasia lacked cytochemically detectable α2,6-linked sialic acid residues but were positive for α2,3-linked sialic acid residues. In contrast, α2,6-linked sialic acid residues were found in colon carcinoma and severe dysplasia irrespective of the histologic type and degree of differentiation. However, the results were based on qualitative observations only. In the present study, densitometric quantitation revealed differentially up-regulated sialoproteins during different stages of oral carcinogenesis. These results are in accordance with Dall'Olio report (2000), which showed that α2,6-sialoproteins levels were closely correlated to the ST6Gal I mRNA expression. Also, the level of α2,6-sialylation of membrane glycoproteins

determined by lectin SNA and *Tricosanthes japonica* were increased in colon cancer (Yamashita et al., 1995; Dall'olio and Trere, 1993; Sata et al., 1991). A strong SNA reactivity has been proposed to be an indicator of a poor 5-year survival (Vierbuchen et al., 1995). Consistent with the notion of an onco-developmentally regulated expression of sialyl-α2,6-lactosaminyl linkage, a down regulation of SNA reactivity upon weaning has been described in rat (Hamr et al., 1993; Taatjes and Roth 1990) and pig intestine (King et al., 1995).

Dot blot analysis for serum sialoproteins revealed significantly elevated α2,6-sialoproteins in untreated oral cancer patients as compared to controls and patients with OPC (**Figure-4.3A & B**). Further, multivariate analysis (**Table-4.4**) illustrated that stage of the disease had significant correlation with serum α2,6-sialoprotein variations. The serum levels of α2,6-sialoproteins in patients with poorly differentiated tumours were higher than well and moderately differentiated tumours **(Figure-4.9)**. No significant difference was observed in the serum α2,3-sialoproteins levels among three groups (**Figure-4.3C & D**) as well as with clinicopathological parameters (**Table-4.4**). Even though the mean TSA and SNA reactivity of cancerous tissue is always higher than that of normal mucosa, in few cancer cases there was a marked discrepancy between the levels of α2,6-SiT and the expected degree of SNA reactivity. As reported earlier, the inconsistent expression of oligosaccharide antigens and the cognate glycosyltransferases are not the uncommon features (Brockhausen, 1993). This indicates that the control of oligosaccharide expression is multi-factorial. One of the mechanisms that may be invoked to explain enzyme/structure inconsistencies is based on the competition between glycosylation pathways. For example, the enzyme involved in SLe^X antigen (2,3-SiT and/or 1,3(4)-FucT) competes with ST6Gal I for the same acceptor substrate. The possibility of a competition between the pathways leading either to the synthesis of SLe^X antigen or α2,6-sialylated N-acetyllactosamine is of special interest in light of the known ability of the two epitopes to interact with different cell adhesion molecules.

Analysis of different forms of SiT along with sialic acid and sialoproteins levels in tissue and serum can be useful for a better understanding of the mechanism of elevations in sialic acid during malignancy. Hypersialylation as the result of enhanced enzyme activity leads to reduce cell-cell adhesion (Bosch et al., 1998; Dall'Olio et al., 1992) and/or increased adhesion of tumour cells to the basal membrane (Morgenthaler et al., 1990), and may hamper immune response mechanisms (Pilatte et al., 1993). Altered sialylation, which occurs during carcinogenesis and metastasis, is associated with either enhanced or decreased different type of SiT activity (Lin et al. 2002; Wang et al., 2002, 2001; Schneider et al., 2001; Zhu et al., 2001; Yamamoto et al., 1997; Hakomori 1996; Harvey et al., 1992; Wagner et al., 1990). SiT studies are interesting because final fate of this reaction altered during malignant transformation because of several reasons: (i) sialic acid can prevent cell-cell interactions through non-specific charge repulsion effects; (ii) sialylated structures can be specifically bound by cell adhesion molecules such as selectin (Feizi T, 2000) or the siglecs (Crocker and Varki, 2001) families; (iii) the addition of sialic acids may mask the underlying sugar structure, thus avoiding recognition by other lectin like molecules, such as galectins (Colin-Hughes, 2001); (iv) the regulatory elements of SiT genes might be a target of specific cell signaling cancer cell might indicate that a specific signaling pathway has been activated. An example is provided by the up-regulation of ST6Gal I by *ras* oncogene over-expression (Easton et al., 1991).

Traditional assays for SiT employ radiolabeled CMPNeuAc (Paulson, 1985) and either glycoprotein or small oligosaccharide acceptors. In addition to the problems associated with the handling of radioisotopes, and the technical difficulties of rapidly counting large numbers assays, the typical assays for SiT also involve difficult task of identifying the product. In biological samples, both α2,3-SiT and α2,6-SiT are present and both can utilize the same acceptor Galβ1-4GlcNAc to generate the respective products, NeuAcα2,3Galβ1-4GlcNAc and NeuAcα2,6Galβ1-4GlcNAc, which detects total SiT activity instead of individual isoforms. To overcome these issues, in the

present study, α2,3-SiT and α2,6-SiT were analyzed using a recently developed absorbance based solid-phase non-radioactive method (Shibuya et al., 1987) using linkage specific plant lectins SNA and MAL, which bind with high affinity to the terminal sequence (Yeh and Cummings, 1996; Knibbs et al., 1991; Wang and Cummings, 1988).

Among other studies, various isoforms of α2,6-SiT and α2,3-SiT mRNA expressions as well as total enzyme activity of SiT have been studied in various malignancies. However, the isoforms of SiT in oral cancer have not been reported so far. The present study evaluated the significance of α2,6-SiT and α2,3-SiT in tissues and serum of oral cancer patients. Furthermore, enzyme activity was estimated from serum of patients with OPC. Paired and unpaired t-test showed significantly elevated α2,6-SiT and α2,3-SiT in oral malignant tissues than their adjacent normal tissues (**Table 4.6; Figure-4.16A & B**). Whereas, serum analysis showed significant high α2,6-SiT activity (**Figure-4.5A**) in untreated oral cancer patients as compared with the controls and patients with OPC. However, the mean serum levels of this enzyme in patients with OPC were lower than the controls. Serum α2,3-SiT levels were comparable between all the three groups (**Figure-4.5B**). It can be inferred from the results that α2,3-SiT have role during initiation of oral carcinogenesis, whereas α2,6-SiT may have important functions in progression and metastatic potentials of tumours. It may be possible that activities of the different isoforms may have different role during the diseases progression. The activities of both the enzymes were increased in tumour tissues and were positively correlated with disease stages. This suggests that tumour cells may escape from the primary site due to increased total sialylation of cell surface proteins. Recchi and co-workers (1998) have found that ST3Gal may be involved in the synthesis of SLe^X ligand. However, they could not establish any clear link between these two markers. The present study observed significant increase in α2,3-SiT activity and overexpression of SLe^X in oral malignant and OPC tissues. The possible reason for the discrepancies between the two enzyme activities may be due to α1-

macroglobulin, which is specifically against α2,6-SiT and competes with neither the donor, nor the acceptor in the enzyme reaction. It behaves with a mixed type of inhibition and may directly entrap the enzyme, disabling its catalytic activity.

The present investigation demonstrated the association of α2,6-SiT and α2,3-SiT activities in serum as well as malignant tissues with different clinicopathological characteristics of tumours. Student t-test analysis revealed that mean α2,6-SiT and α2,3-SiT activity were increased in tumour tissues with disease progression from stage I to stage IV (**Figure-4.18A & B**). Tissue levels of α2,3-SiT were also increased with the decrease from well to poor differentiation of tissues **(Figure-4.19)**. The multivariate analysis exhibited significant association between serum α2,6-SiT activity and nuclear grade III tumours (**Table-4.4**). Also, oral cancer patients with advanced stage of disease (stage III + IV) showed significantly enhanced serum α2,6-SiT (**Figure-4.8; Table-4.5**) than the early stage of the disease (stage I + II). The association of these enzyme activities with different histopathological parameters of oral cancer may suggest their potent role with disease progression. It has been shown that high ST6Gal I mRNA expression is associated with poor histopathological parameters such as grade III has poor differentiation and the absence of a progestron receptor (Recchi et al., 1998), high invasive potential (Wang et al., 2001; Dall'Olio et al., 2001, 1999; Pousset et al., 1997; Bergler et al., 1997), and lymphnode metastases (Wang et al., 2002), especially in solid tumours such as colorectal cancer, hepatocellular cancer and SCC of cervix, although some results showed conflicted data, especially for tumours from lymphoblastoid cell origin (Lo et al., 1999). ST6Gal I mRNA expression and SiT activity correlated well in cervical cancer and normal specimens (Wang et al., 2002). SiT mRNA expressions of ST6Gal and ST3Gal has been reported in various malignancies (Wang et al., 2003, 2002, 2001; Dall'Olio et al., 1999; Recchi et al., 1998; Bergler et al., 1997; Whitehouse et al., 1997). Moreover, the changes in transcriptional regulation of ST6Gal-I expression is through tissue-specific

promoters whose usage results in the production of mRNA species which diverge in the 5' untranslated regions have been observed in cancerous tissues and cells (Dall'Olio et al., 1999; Dall'Olio 2000; Taniguchi et al., 2000; Lo and Lau, 1996). Therefore, it was interesting to explore the expressions of specific SiT isoforms in oral cancer.

The goal for cancer research during the next few years will be elucidation of the precise molecular interactions involving carbohydrate chains in the hope that this will facilitate the design of drugs directed against specific steps of cancer progression. The use of glycosylation inhibitors such as swainsonine, as antineoplastic agents or attempts to use the sialyl-Tn epitope for active anticancer immunotherapy, are promising new approaches for the treatment of cancer (Holmberg and Sandmaier 2001; Dall'Olio, 1996). Recent development in this field has focused in designing the carbohydrate mimetics and the structure-activity relationships of substrate-based SiT inhibitors. This may prove usefulness of inhibition of SiT in elucidating the biological functions of sialylation. Also, carbohydrate mimetics can be potentially valuable as effective tools for glycobiology research, and as new potent anti-inflammatory, immunosuppressive and antimetastatic agents for future therapeutic applications (Wang et al., 2003). Furthermore, antisense treatment of glycoprotein-specific transferase, which led to a decreased Gal(β1-4)GlcNAc(α2-6)-SiT activity on the level of protein expression as well as on the mRNA level with specific oligonucleotides complementary to the region upstream of the initiation codon (Kemmner et al., 1997). Antisense treatment has no effects on cell viability or cell growth. Thus, this appears as a possible way to reduce cell surface sialylation, particularly on the tumour cells. The prospects for the further development of SiT inhibitors are very promising.

Another key enzyme of sialic acid metabolism is sialidase, which regulates the cellular sialic acid contents in many biological processes. Sialidase levels consistently fluctuate with cell differentiation, cell growth and malignant

transformation, however, very little known about its mechanism and significance. Several observations based on bacterial sialidase treatment on cancer cells suggested that altered endogenous sialidase activity might be related to tumorigenic transformation and tumour invasiveness (Bosmann and Hall, 1974). The results of current work demonstrated that sialidase activities in malignant tissues were significantly higher as compared to their adjacent normal tissues (**Table-4.6; Figure-4.17**). Interestingly, when tissue sialidase activity compared between different stages of the disease, the levels were decreased significantly with increase in the stage of the disease (**Figure-4.18C**), which inversely correlated to the SiT activity. Multivariate analysis also showed significant correlation of serum sialidase activity with the advanced stage of the disease (stage III+IV vs. stage I+II), nuclear grade and tumour differentiation (**Table-4.4**). These results suggested that the elevated levels of tissue sialidase might be caused by an increase in its synthesis or its activity in tissues to compensate the effect of hypersialylation by SiT. Also, the rise in serum sialidase concentrations may be due to the necrosis of tumour tissue. However, decrease in enzyme activity with extent of the disease from stage I to IV indicate that decrease in activities may have a role in tumours aggressiveness and poor prognosis of oral cancer patients. Reuter et al. (1992) have found higher sialidase activities in oral secretions of the patients with upper aerodigestive tract than the normal glands. They have hypothesized that tumour itself may contribute for high enzyme activity in secretions, leading to a decrease in mucin-bound sialic acid and subsequently to a reduced protective function of the mucin thus facilitating the tumour formation and progression. Sonmez and colleagues (1999) have found significantly higher serum and tissue sialidase activity in breast cancer patients as compared to controls. Furthermore, elevations in serum and tissue sialidase activity in patients with grade III tumours compared to grade I and II were observed. The studies reported by Rothenberg and co-workers (1994, 1996) have demonstrated low blood sialidase levels in breast cancer patients. The authors have shown that inadequate activity of sialidase may be a marker and predisposition toward increased risk of breast cancer.

Sawada et al. (2002) demonstrated that overexpression of sialidase activity in mouse colon adenocarcinoma cells results in a decreased metastatic ability. It is not dependent on a reduction of the overall sialylation levels but is dependent on a reduced expression of specific sialylated structures, such as SLe^X and ganglioside GM3. Interestingly, overexpression of SLe^X and decrease in sialidase activity with increased stage of the disease in oral tumour tissues was observed in this investigation. In contrast, Miyagi et al. (2004) have indicated that the high expression of plasma membrane associated sialidase in cancer cells leads to protection against programmed cell death.

Theoretically, sialic acid metabolism revealed that increased SiT leads high sialylation resulting into elevated levels of sialoproteins in the biological samples. These events direct the cells to increase the production of sialidase, hydrolytic enzyme of sialic acid metabolism, to prevent any cellular disorder. Thus, all these molecules regulate biological functions of cells. However, the exact biological phenomenon of cell regulation is not completely understood. In the present study, Pearson's correlation analysis (**Table-4.2**) revealed that serum α2,6-SiT activity was positively correlated with TSA and α2,6-sialoproteins levels, but negatively correlated with sialidase activity. Similarly, α2,3-SiT activity in serum was also positively correlated with α2,3-sialoproteins levels and negatively correlated with sialidase activity. It is important to note that α2,6-SiT and α2,3-SiT activities in serum showed inverse correlation. Malignant tissues also showed similar trend (**Table-4.8**). Further, serum α2,6-SiT and α2,3-SiT activities showed negative correlation with sialidase activities in patients with OPC. A positive correlation was observed between serum levels of TSA and α2,6-SiT activity **(Table-4.3)**. Thus, the results support that overall hypersialylation of oral tumour cell surface provide a high negative charge to increase cell-cell repulsion and facilitate the tumour cells to escape from primary growth.

5.3: ALTERATIONS IN CIRCULATING AND TISSUE LEVELS OF FUCOSE, FUCOPROTEINS AND FUCOSIDASE

Fucose is second important terminal sugar plays crucial role in various cellular functions. Aberrant fucosylation is the frequent phenomena associated with oncogenic transformation (Alhadeff, 1989; Hakomori, 1989). The diagnostic and prognostic significance of serum fucose levels for various malignancies have been reported (Patel et al., 1994; Wang et al., 1995). However, studies on fucose levels in oral cancer patients as well as patients with OPC are not described. The present study examined that the serum fucose levels (**Figure-4.21A**) in oral cancer patients were significantly higher as compared to the controls and patients with OPC. Patients with OPC also showed significant elevations in serum fucose levels than the controls. The results are in agreement with previous reports (Rao et al., 1998; Shashikanth and Rao, 1994; Ghosh et al., 1991), however, none of the previous studies have reported fucose levels in patients with OPC also the number of oral cancer patients were less. Serum fucose contents when normalized to serum total proteins (fucose/TP) also showed similar trend in the patients (**Figure-4.21B**). This indicates increase in serum fucose is independent to proteins alterations. Recently, it has been shown that fucose is a relevant part of several ligands involved in adhesion processes; it is not surprising that additional pathological recognition events are dependent on fucosylated glycans (Listinsky et al., 1998). Fucose-containing lewis antigens $Le^{a/b/x/y}$, sialylated lewis $SLe^{a/x}$ antigens and H-antigens are found to be expressed in a number of human adenocarcinomas as well as in Hodgkin's disease, leukemia, and malignant melanomas. Evidence for fucose-containing tumour associated carbohydrate antigens studied to date, suggest that fucose is the critical sugar to mediate binding of neoplastic cells to E-selectin of endothelium. This is one of the key steps in metastasis via hematogenous routes. Fucosylation changes also play an important role during apoptosis (Russell et al., 1998). Multivariate analysis (**Table-4.10)** showed significant association of serum fucose and fucose/TP with tumour differentiation. Further, the comparison of mean serum fucose and fucose/TP in patients with oral cancer were

significantly increased as the degree of differentiation decreased from well to poorly differentiated tumours (**Figure-4.28**). Earlier report by Wallack et al. (1978) also established an association between elevated serum fucose levels and poor prognosis in breast cancer patients.

Lectins have been used as important tools for exploring the structure and functions of carbohydrates of glycoproteins (Montreuil et al., 1997). Currently, the use of lectins for the investigation of carbohydrates, could contribute tremendously to the advancement of glycobiology. In this study, serum fucoproteins were isolated using specific lectin *lotus tetragonolobus,* its specificity is directed towards fucose that linked either α1,2 or α1,6 position in core or α1,3 position as an external residue. The electrophoretic patterns in the study clearly indicated significantly higher fucosylation of serum proteins, especially the ~43 kD and ~66 kD proteins (**Figure-4.22 & 24**), in untreated oral cancer patients as compared to the controls and patients with OPC (**Table-4.9**). The expression of fucoproteins appeared to broadly reflect increase in total fucose, which may be due to the hyperfucosylation of the 43 kD and 63 kD region serum proteins. These results are similar to Thompson's observations (Thompson et al., 1991; Thompson and Turner, 1987) in sera of different malignancies. The glycomics approach can be employed for further characterization of these fucoproteins. Serum fucoproteins also revealed clinical importance as treatment monitors for oral cancer patients (**Figure-4.23A & B, 4.25; Table-4.9**). Shirahama et al. (1993) have shown that the degree of fucosylation of tumour proteins in urinary bladder cancer patients reflected the metastatic potential and poor survival. The observations were done on the basis of qualitative immunohistochemical analysis of fucoproteins. Thus, the results of the present study become more precise and important which provided densitometric quantitation of fucoproteins.

The turnover of fucose residue in glycoconjugates is achieved by the implication of FucT and fucosidases. The fucoproteins levels in malignant cells are associated with the FucT activity. A marked reduction in fucoprotien

content in colon tumour tissues with a concomitant reduction in FucT activities compared with the normal tissues has been reported (Takahashi et al., 2000; Togayachi et al., 1999; Wang et al., 1995). α1,6-FucT is responsible for fucosylation of α-fetoprotein, an important marker for hepatocarcinogenesis (Noda et al., 2002). Increased activities of FucT lead hyperfucosylation that reflects as elevated levels of fucoproteins during neoplastic transformation.

On the other hand, catabolic enzyme of fucose metabolism α-L-fucosidase is also important for neoplasia. The present study provided evidence of α-L-fucosidase in tissues and serum of patients with oral cancer and patients with OPC. The increased expression of tumour-associated fucose-containing glycoconjugates, cannot be explained by decrease in α-L-fucosidase activity since this degradative enzyme showed significant enhanced activity in tumour homogenates of the patients in the present study (**Figure-4.30A & B**). However, no association could be established with histopathologic parameters. This observation is in agreement with the results by Wang et al. (1995) who found an increased α-L-fucosidase activity in endometrial cancer tissues. Fernandez-Rodriguez et al. (2000) have evaluated α-L-fucosidase activity in primary tumours as an independent prognostic factor of tumoural recurrence. To understand the pattern of recurrence of tumours and the features that predispose to them by knowing a biochemical parameter may guide physicians in aggressive but more selective adjuvant therapy and recommendations for targeted surveillance in follow-up. In the current study, serum fucosidase activities were significantly elevated in patients with oral cancer and patients with OPC as compared to controls (**Figure-4.27**). Other investigators have also found association of increased serum α-L-fucosidase with colorectal, endometrial, breast, cervical, ovarian and hepatocellular carcinomas (Girdina et al., 1998; Abdel-Aleem et al., 1996; Takahashi et al., 1994). Alterations in serum/plasma α-L-fucosidase activity have been proposed to have utility in diagnosis, prognosis, early detection of recurrence, as well as management of colorectal cancer (Ayude et al., 2003; 2000). The

enzyme has been documented as a marker for development of hepatocarcinoma (Girdina et al., 1998). The present study showed significant correlation of serum fucosidase activity with the extent of disease (**Table-4.10**), which may indicate association of the enzyme activity with tumour progression. Moreover, Ayude et al. (2003) found that patients with higher serum enzyme values had a worse prognosis and measurement of preoperative serum α-L-fucosidase activity in colorectal carcinoma could serve to detect hidden metastasis.

5.4: USEFULNESS OF SIALIC ACID AND FUCOSE ASSOCIATED PARAMETERS AS TREATMENT MONITORS IN ORAL CANCER PATIENTS

Once the malignant disease is diagnosed and anticancer therapy is administered, it is essential to have blood-based test to monitor disease status and subsequently for the management of the patients. Hence, the comparison of the pre-treatment and post-treatment levels of markers may help to monitor the patients' response to anticancer therapy. Therefore, in this retrospective study, the usefulness of sialylation and fucosylation markers was determined in predicting treatment response. The parameters were also analyzed from the follow-up serum samples collected from the oral cancer patients during and/or after anticancer treatment and compared with their PT levels.

Paired 't' test analysis showed that mean serum TSA, TSA/TP, fucose, fucose/TP and fucoproteins as well as activity of α2,6-SiT and α2,3-SiT and α-L-fucosidase were significantly declined in CR compared to PT levels (**Figure-4.10, 4.22; Table-4.11, 4.9**). While, sialidase activities were not significantly lower in CR then PT values. These results indicate that the decrease in the markers was suggestive of favourable treatment outcome. The comparison of the serum levels between PT and NR showed that the markers were either elevated or comparable between the two groups (**Figure-4.11, 4.24; Table-4.12, 4.9**). Moreover, the serum levels were also compared between CR and NR using unpaired student 't' test, which

revealed declined marker levels in CR as compared to NR. The NR group included all the patients with partial or no response to anti-cancer treatment. These results are in accordance with previous reports from this laboratory (Raval et al., 2003) as well as others (Waters et al., 1992; Nakata et al., 1998). However, the complete marker panel is not evaluated earlier in oral cancer as treatment monitors. The results clearly indicated that markers reflected patients' response or failure to the treatment in terms of decrease or increase in the serum levels during each follow-up durations (**Figure-4.12A-H, 4.23A, B, 4.25, 4.29A-D**). The response failure (NR group) could be due to several reasons; (i) tumour recurrence and/or disease progression because of locoregional failure which is a major problem after successful operation in patients with oral squamous cell carcinoma (ii) irregular follow-up due to unawareness of the patients. The exact mechanism of alterations in the serum marker levels with anticancer treatment remains vague. However, it is postulated that the decreased circulating marker levels in CR may be due to surgical resection of tumour resulting into decrease in the secretions or shedding of the tumour components.

5.5: USEFULNESS OF SERUM PROTEIN PROFILING

The human plasma proteome holds the promise of a revolution in diagnosis and therapeutic monitoring of cancer provided that major challenges in proteomics and related disciplines can be addressed. Plasma is not only the primary clinical specimen but also represents the largest and deepest version of the human proteome present in any sample. In addition to the classical "plasma proteins", it contains all tissue proteins (as leakage markers) plus very numerous distinct immunoglobulin sequences and the rarest proteins now measured clinically. Although the restricted dynamic range of conventional proteomics technology (2D-PAGE and mass spectrometry) has limited its contribution to 289 proteins that have been reported in plasma to date (Anderson and Anderson, 2002). Only a handful of proteins are currently used in routine clinical diagnosis. The large discrepancy between the expectations arising from proteomics and the realities of clinical diagnostics

should be met. This warrants for the approaches by which protein-disease associations may be more effectively translated into diagnostic tools.

A distinctive finding of the present study, in patients with oral cancer and patients with OPC, was appearance of an unusual serum protein band in the post beta region (**Figure-4.31**). Firstly, the mobility of unknown protein was compared with protein molecular weight standards. It revealed that the retardation factor (Rf) value of unknown protein was comparable to 272 kD standard protein. However, molecular weight estimation in native state is not precise because both charge and mass are the governing forces for electrophoretic mobility. This motivated to match the electrophoretic mobility with the known serum proteins. The electrophoretic pattern revealed that the presence of this protein was between beta and gamma globulin regions of serum profile. Interestingly, the protein found to be glycoprotein in nature as confirmed by Periodic Acid Schiff (PAS) staining (**Figure-4.32**). Further, the gels pre-run on Native-PAGE in tube with and without unusual serum protein band were run on 2D-PAGE. Two distinct spots in CBB stained 2D-map of serum with positive for unusual protein were observed when compared with negative for this protein (**Figure-4.33**). Whereas, the purified elute of the unusual protein showed three bands, when run on SDS-PAGE and stained with more sensitive silver staining method (**Figure-4.34**). Molecular weight determination revealed 54.95, 79.43 and 120 kD apparent molecular weights, respectively (**Figure-4.35; Table-4.13**). Only two spots observed in 2D-map might be due to less sensitivity of CBB stain as well as the minute amount of the unusual proteins in serum. According to the literature, two types of proteins can show overlapping mobility with the band in Native-PAGE i.e. between beta-2 and gamma globulins. One is fibrinogen with molecular weight 340 kD and the second is IgA with molecular weight 160 kD monomer unit. The possibility of fibrinogen was ruled out as the samples were serum and not plasma and there was no fibrinogen clot formation in the sera after the addition of calcium chloride. However, the possibility of IgA cannot be ruled out at present because it may exist as dimeric (mol wt. 320 kD) or

trimeric (mol wt. 480 kD) forms along with some secretary proteins (Varley, 1984).

The presence of an unusual protein band was seen in oral cancer patients, patients with OPC and controls. The protein was more frequently found in oral cancer patients (72%) and patients with OPC (75%) than the controls (24%). The band density was quantified as OD/mm^2 and percentage of band volume were calculated. Though the band was present in all the groups its percentage band volume (in terms of quantity) was higher in oral cancer patients (1-4%) as compared to patients with OPC (0.5-2%) and controls (0.5-1.2%) to total serum protein. The reason for high frequency of this band in patients with OPC and oral patients is not known so far. But, we predict that it may be associated with tobacco habits in the individuals. Further, the healthy individuals showing presence of the extra band may probably be at a higher risk of oral cancer. The prediction may be true in background to the notion that all tobacco users do not develop cancer but majority of the oral cancer patients consume tobacco.

Alterations in glycoprotein electrophoretic pattern chiefly reflect the glycosylated proteins in various fractions. Cancer is accompanied by increased sialylation (α-1 acid glycoprotein, transferrin) and increased fucosylation (α-fetoprotein, haptoglobin) (van-Dijk et al., 1994; Turner, 1994, 1992). Despite the fact that protein bound sialic acid is increased in cancer (Osawa and Tsuji, 1987); the glycosylation of individual serum proteins in cancer has been poorly investigated. This may be due to the complex nature of glycoproteins and lack of specific techniques for its characterization. Also, sialic acid and fucose specific lectins were applied to identify sialoprotein or fucoprotein nature of this protein in the present stdy. However, satisfactory observations could not be drawn which might be due to very less amount of protein in the elute fraction. The current method employed for glycoprotein detection can detect subtle changes in protein glycosylation in pathological sera at various steps of multistage carcinogenesis. Being a simple, less expensive and reproducible approach, it may be used as a routine parameter for

identification of predisposed individuals (who are more likely to develop cancer) among the tobacco consumers. This observation needs to be confirmed with further characterization such as amino acid sequencing and matching with protein data bank, which may provide a new avenue for establishment of marker for identifying high-risk individuals.

5.6: SIALYL LEWIS-X AND E-CADHERIN EXPRESSIONS IN MALIGNANT/OPC TISSUES

Cancer is increasingly recognized as a disorder resulting from abnormal genetic regulation. The major problem in cancer is metastasis, the phenomenon whereby cancer cells leave their tissue of origin and migrate to distant sites through the bloodstream and grow there in an unregulated manner causing catastrophic results. The process of metastasis is an early event in tumorigenesis. The acquisition of metastatic trait may require additional changes beyond those related to tumour growth. Thus, tumorigenicity and metastatic potentials have overlapping and separate features and a group of coordinated cellular processes is responsible for metastasis. Invasion and metastasis are the greatest obstacle to successful anticancer treatment. Although, advances have been made in conventional tumour therapies and surgical techniques, most cancer deaths still result from metastatic disease. The lack of understanding of the molecular mechanisms involved in tumour cell invasion and metastasis has hindered the development of effective anti-metastatic therapies. Therefore, the field of metastasis research needs to be explored to a grater depth.

Metastatic potentials of the tumours are significantly influenced by the local microenvironment and its molecular phenotype. Several critical events in this metastatic cascade depend on cell-to-cell or cell-to-matrix interactions that involve cell surface molecules especially, glycoproteins. Thus, alterations in glycoprotein moieties can affect a variety of processes in normal as well as malignant cells. Oligosaccharide moieties of cell-surface glycoproteins are thought to be involved in recognition events during invasion and metastasis.

The current problems with oral cancer presentation need biomarkers, which could be useful to predict better prognosis of the patients.

One of the promising cell surface molecules SLe^X plays key role in number of biological interactions (Cooling et al., 1997). SLe^X were originally identified as human tumour-associated antigens and are found on highly malignant types of cancer cells. In the present work, significantly high expression of SLe^X was observed in oral tumours as compared to their normal counterparts (**Table-4.14; Figure-4.37A**). The approximate band size of protein contains SLe^X epitope is 200 kD (**Figure-4.36**). SLe^X was also analysed in ten OPC tissues. Interestingly, OPC tissues showed high SLe^X expressions than their adjacent normal tissues (**Table-4.14; Figure-4.37B**). The presence of SLe^X in OPC tissues could be an early event during oral cancer progression. The SLe^X expressions in adjacent normal tissues may be suggestive of the process of field cancerization (Saranath, 1999). The apparently adjacent normal tissue may have undergone certain genetic and biochemical changes even though these cells may yet be histopathologically normal or certain malignant cells might have spread to the adjacent normal area from the tumour site. This suggests an early event or the presence of occult malignant disease even after surgical resection of the tumour. However, local recurrence may occur even with histopathologically negative surgical margins (Vikram, 1984). Hence, comparison of malignant tissues with their corresponding adjacent normal tissues will allow understanding of activation of malignant phenotypes. The presence of SLe^X on cancer cells induces the expression of E-selectin on endothelial cells and serves as an efficient ligand during hematogenous metastasis. Hanski et al. (1993, 1995) have reported that mucins MUC1 and MUC2 serve as carriers of SLe^X in colon cancer. In support of this, Mann and colleagues (1997) have suggested that some unidentified mucins may carry significant amount of this carbohydrate epitope. However, other comparative study indicated MUC1 and SLe^X as independent prognostic parameters of colon cancer (Baldus et al., 2002). The increased accessibility of SLe^X epitopes in carcinoma is reported to be due to the decrease in O-acetylation of the

sialic acid residues (Jass et al., 1995; Ogata et al., 1995). Several laboratories have reported that expressions of SLe^X are increased substantially in tumour cells such as carcinomas and leukemias (Shimodaira et al., 1997; Saitoh et al., 1992; Fukuda et al., 1985; Fukushima et al., 1984). SLe^X has been used as serum marker for diagnosis of cancer (Ørntoft and Bech, 1995). It has been reported that B16-F1 melanoma cells become highly metastatic by acquisition of SLe^X through expression of fucosyltransferase III. Moreover, they demonstrated that tumour formation was inhibited by pre-incubation of those cells with anti-SLe^X antibody but not by incubation with a control antibody specific to Lewis X.

Present study showed significant association of SLe^X expressions with clinicopathological parameters. SLe^X was significantly high in nuclear grade III poorly differentiated tumours than the nuclear grade I or II with well or moderately differentiated tumours (**Figure-4.41 & 4.42**). High expressions was observed in patients with lymph node metastasis and advanced disease (stage III and IV) as compared to the patients negative for lymph node metastasis and early disease (stage I and II) **(Figure-4.43 & 4.44)**. These observations are similar to the previous reports in breast and colonic carcinoma patients. The presence of SLe^X and SLe^a was correlated well with poor prognosis (Renkonen et al., 1997; Shimodaira et al., 1997; Nakamori et al., 1993). A study demonstrated that expression of SLe^X and SLe^a in mucin-type *O*-glycans is highly correlated with lymphatic and venous invasion (Shimodaira et al., 1997). Previous reports on SLe^X expressions in colorectal and non-small cell lung cancer has been correlated with advanced disease, increased incidence of distant metastasis and decreased survival (Ogawa et al., 1994; Nakamori et al., 1993). An *in vitro* study has identified molecules capable of interfering with metastatic process by inhibiting SLe^X-mediated adhesion between tumour cells and activated endothelial (Fukami et al., 2002). Apart from the use as tumour marker, antibodies raised towards SLe^X may be used as a passive tumour vaccine. Several reviews have appeared on

the role of SLe^X and its analogs in cancer treatment (Kannagi, 1997; Hakomori and Zhang, 1997).

The cadherins are a major class of adhesion molecules that play an important role in the homotypic cell-cell adhesion and, hence, in cancer cell invasion and metastasis. E-cadherin, a member of cadherin family is expressed on all epithelial cells. Being cell surface protein, E-cadherin is glycosylated. Przybylo et al. (2002) have documented that N-glycosylation pattern of cadherin in a bladder cancer cell line undergoes modification during carcinogenesis. It has been suggested that the E-cadherin system could be inactivated by multiple mechanisms in cancer, including both genetic and epigenetic events (Hirohashi, 1998). However, the mechanisms of functional loss of E-cadherin expressions have not been fully understood, whether it occur at genetic levels or due to the abnormal synthesis of this protein. Few mechanisms have been proposed for alterations in E-cadherin expression or function in tumour progression. The cytoplasmic domains of E-cadherin interact strongly with a group of intracellular proteins known as catenins; which are essential for their functions. The truncation of the E-cadherin cytoplasmic domain deletes catenin binding sites leads to a loss of cadherin-mediated adhesion (Gumbiner, 1996; Takeichi 1995).

The present study demonstrated E-cadherin in tissue samples detected by western blot using HECD-1 antibody. The reactivity of the antibody was observed with two bands at ~120 kD and ~97 kD sizes (**Figure-4.38**). The presence of ~97 kD E-cadherin was termed as post-translationally truncated protein. The significant difference was observed between tumour as well as OPC tissues and their adjacent normal tissues with respect to the relative cleaved/truncated soluble $E\text{-}cad^{97}$ (**Table-4.15**). Paired t-test analysis confirmed a relative increase in the truncation in the tumours and ten OPC tissues as compared to the normal tissues of the same patient (**Figure-4.39A & B**). Also, few adjacent normal tissues showed presence of $E\text{-}cad^{97}$, which may indicate the occult disease in the surrounding tissues. In respect to $E\text{-}cad^{120}$ expressions, no significant changes between tumour and adjacent

normal tissues were observed. The results indicate that newly synthesized E-cadherin by tumour cells may be truncated. Although, equal amount of protein lysates were loaded for each sample, the actual percentage of epithelium may vary depending on the relative amount of stroma present. Therefore, the ratio of E-cad^{97}: E-cad^{120} was calculated to establish a value that is independent from the epithelial and stromal distribution. The significant increase in the ratio of the truncated to the native E-cadherin in the tumour relative to its case matched normal tissue was observed. The truncation of E-cadherin leads to the relative loss of β-catenin binding domain in tumour, by increased accumulation of E-cad^{97} that is not present in the β-catenin functional complex. This may provide an alternative mechanism for the loss of E-cadherin function. The findings of this study in oral cancer are in accordance with a recent report by Rashid et al. (2001). They were the first to report E-cadherin truncation in prostate tumours. Also, Gofuku et al. (1998) have found elevated levels of soluble E-cadherin in patients with gastric cancer. Earlier studies have examined E-cadherin expression by immunohistochemical analysis from processed tissue sections. However, immunohistochemistry using HECD-1 anti-body cannot distinguish between the full-length E-cad^{120} and truncated E-cad^{97}.

An essential factor for the success of clinical therapy of oral cancer is to suppress lymphnode metastasis; therefore it is important to predict the metastatic ability of primary tumours, as the detachment of the cancer cells from the primary tumour is the initial step in the metastatic process (Nicolson, 1982; Hart and Fidler, 1981; Fidler, 1978). E-cadherin plays a major role in metastatic process (Frixen et al., 1991) since impaired expression of E-cadherin is frequently observed in tumours having a high degree of invasion and lymphnode metastasis (Oka et al., 1993; Shiozaki et al., 1991; Takeichi, 1988). However, a much larger fraction of cancer showed reduced or absent E-cadherin mRNA or protein expression, yet no mutations in E-cadherin gene could be detected (Kanai et al., 1997). Several experimental studies have reported that CpG methylation around the promoter region may be associated

with a possible mechanism of the E-cadherin gene silencing in oral cancer (Nakayama et al., 2001; Saito et al., 1998) and other malignancies (Chen et al., 2003; Tamura et al., 2000; Melki et al., 2000; Graff et al., 1998). Thus, it is possible that newer therapeutic strategies can now be developed based on re-expression of gene products by targeted gene delivery approach.

A key determinant in tumour progression is the ability of cancer cells to survive and proliferate in the absence of extracellular contact. In tumours of epithelial origin, the loss of adhesion dependence may rise through alterations of E-cadherin-mediated pathways. It has been reported that E-cadherin truncation and inactivation in cells destined to undergo apoptosis (Vallorosi et al., 2000; Day et al., 1999). Whereas, Rashid et al. (2001) postulated that accumulation of E-cad^{97} may reflect early apoptotic signaling events resulting from the breakdown of adhesive interactions. However, these cells may be detached and are unable to execute apoptosis because of downstream mutations or inactivating events. Therefore, the truncated species may be accumulating in tumour cells that are not undergoing apoptosis. Another mechanism put forward is that matrix metalloproteinases (MMPs) play significant role in proteolytic degradation of E-cadherin. The resultant ectodomain shedding of E-cadherin might have an active role in tumour progression via extracellular matrix degradation by upregulating MMP-2, MMP-9 and MMP-14 (Nawrocki-Raby et al., 2003). Barbletz and co-workers (1999) have documented an indirect evidence of E-cadherin involvement in tumour progression and reported that accumulation of β-catenin leads to activation of MMP-7 in colon tumours.

Earlier studies have suggested that E-cadherin has potentials as a prognosticator in different malignancies (Siitonen et al., 1996; Lipponen et al., 1994; Giroldi et al., 1993). Elzagheid et al. (2002) have reported that the survival of lymphnode-positive breast cancer patients can be predicted by E-cadherin index. They have also found that post-menopausal patients with E-cadherin positive tumours have an increased survival. Chan et al. (2003; 2001) have correlated high concentration of serum soluble E-cadherin with

inoperability/ palliative treatment and lymphnode metastasis as well as potentially valuable pre-therapeutic prognostic factor in gastric cancer. In contrast, Knudsen et al. (2000) did not observe any correlation of serum E-cadherin with the presence of breast cancer. In the present study, significant correlation of truncated E-cad^{97} with various clinicopathological parameters in oral cancer patients was observed. High expression of E-cad^{97} and E-cad^{97}: E-cad^{120} was seen in patients tumour tissues with lymphnode-positive as well as advanced disease as compared to the patients with lymphnode-negative and early disease, respectively (**Figure-4.43 & 4.44**). Similarly, poorly differentiated grade III oral tumours showed high expression of E-cad^{97} and ratio of E-cad^{97}: E-cad^{120} (**Figure-4.41 & 4.42**). However, native E-cad^{120} expressions did not show significant correlation with various histological parameters. The results clearly indicate the association of loss or truncation of E-cadherin with disease progression and worse prognosis in oral cancer patients.

In addition to their adhesive functions, cell-adhesion molecules modulate signal-transduction pathways by interacting with molecules such as receptor tyrosine kinases, components of the WNT signaling pathway and RHO-family GTPases. Thus, changes in the expression of cell-adhesion molecules affect not only the adhesive repertoire of a cell, but also its signal transduction status, which makes these molecules more fascinating among researchers. Conversely, signaling pathways can modulate the function of cell-adhesion molecules, altering the interactions between cells and their environment. Recent experimental evidence indicated that such processes have a crucial role in tumour progression, in particular during invasion and metastasis (Noren et al., 2003; Bienz and Clevers, 2000; Polakis, 2000).

The ROC curve (**Figure-4.40**) revealed that SLe^X as well as E-cad^{97} and ratio of E-cad^{97}: E-cad^{120} have good discriminatory efficacy with high sensitivity and specificity between oral malignant and adjacent normal tissues. The area under the curve for SLe^X as well as E-cad^{97} and ratio of E-cad^{97}: E-cad^{120} were 0.83 (p=0.001), 0.70 (p=0.042) and 0.78 (p=0.004) respectively (**Table-**

4.16), indicating a high accuracy. Thus, the results indicate that these parameters can serve as markers for oral cancer progression.

5.7: IMPORTANCE OF TERMINAL GLYCOSYLATION IN ORAL CANCER

The new era raises new challenges for translating the new discoveries to the clinic. It is likely that the paradigm of treatment will now move from "seek and destroy" to "target and prevent". Genomics have contributed greatly to the understanding of the molecular basis of disease. However, only genome sequence does not help to understand its functions. To address this issue, the field of proteomics has emerged with the global analysis of proteins expression and function. The proteins, however, are not the final gene products in many cases. It has been shown that carbohydrates participate in post-translational modifications and in many other functional regulations; hence, the study of the glycome is essential in order to determine the functions of all gene products. The structural diversity of glycans caused by numerous linkage isomers and branching events is far greater than that of nucleic acids and proteins. Though, it is clear that glycans are fundamental to many biological processes, their biological functions are still not completely explored. It is one of the most important determinants for functional classification of proteins and taken into account when deciphering protein function and characterizing complete proteomes (Mechref and Novotny, 2002).

Progress during the last two decades of research on cellular glycoconjugates justifies adding the enzymatic production of glycan antennae with information-bearing determinants to this famous and basic pathway. There are varieties of regulatory functions ranging from cell growth, cell-cell interactions and adhesion/migration have been unraveled and are mediated or modulated by specific protein-carbohydrate interactions. Nevertheless, potentially hundreds of enzymes involved in oligosaccharide biosynthesis, a direct sequencing approach remains daunting. Integration of constantly improving molecular biology techniques with emerging substrate-based and

genomic wide approach promises rapid progress in determining the molecular forces that govern oligosaccharide biosynthesis. The unsurpassed ability of the sugar code (glycocode) to store biological information in short oligomers is crucial for this role as the third dimension of molecular biology. The perspective for medical applications summarized in the following sentence: "Breaking the glycocode and identifying the receptors are of prime importance not only for theoretical reasons, but also to facilitate the development of novel treatments for the many diseases in which carbohydrate recognition plays a key role" (Sharon, 1998). Also, carbohydrate-based cancer treatments aim to alert the immune system to the tumour's glycan disguise. The researches and biotechnology companies are now developing anticancer vaccines that stimulate an immune response against the carbohydrate antigens found on tumour cells. Although these antigens are normally tolerated by the immune system, evidence is emerging that chemically modified glycans and adjuvants can boost immunity, causing tumours to be held in check or possibly even eliminated. Thus, for biotechnology companies, glycobiology has become an area really ripe for potential therapeutics intervention.

To summarise, the present investigation was focused on understanding the significance of terminal glycosylation changes in patients with oral cancer and OPC. The results successfully unmasked several important aspects of oral carcinogenesis in relation to glycoprotein changes. Increased levels of the terminal sugars and related proteins and enzymes in patients with OPC may indicate early events during malignant transformation and thus would help in identification of high risk group. The variations in the sialylation and fucosylation pattern of serum and tissue proteins would provide data about malignant potentials of tumour at the time of diagnosis. Further, appearance of unusual serum protein band may provide avenue for identifying high risk individuals among the healthy population early changes and during oral cancer progression. Expressions of SLe^X and truncated $E\text{-}cad^{97}$ are found to be associated with alterations in cell adhesion and in-turn invasion and/or

metastatic properties of cancer cells. Inactivation of E-cadherin can possibly due to aberrant translational regulation or posttranslational events dysfunction, which would be a remarkable approach to predict the prognosis of the patients. To put all the observations together, the study demonstrated remarkable association of sialylation and fucosylation alteration as well as over expression of truncated E-cadherin and SLe^X with oral cancer. Alterations in terminal glycosylations were observed in oral precancerous stage and also have correlation with different stages of malignant disease along with other histopathology, which may help in detection of early changes and prognostication of oral carcinogenesis. The serum markers could be useful in treatment monitoring and management of cancer patients. The markers could also be useful for predicting relapse free survival and recurrence of the disease. Thus, The present findings strengthen the significance of glycoproteins in oral carcinogenesis in the era of Proteo-Glycomics.