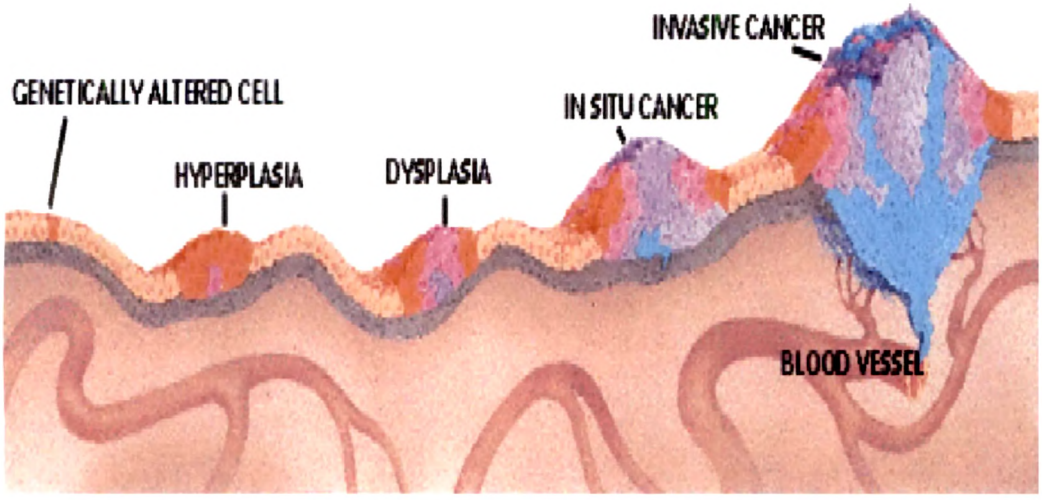
Prologue



Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

- Curie, Marie

1. PROLOGUE

In the 21st century, the numbers of individuals at cancer risk are increasing rapidly. This dreaded disease is the second major cause of death in the world after cardiovascular disorders. It remains a major public health challenge despite progress in detection and therapy and exerts a tremendous toll on the society. In addition to the devastating effects on patients and their families, the economic costs of cancer are enormous, both in terms of direct medical-care resources for its management and in form of loss of human capital due to early mortality. The causes of cancer are many and diverse which include genetic pre-disposition, life style factors environmental influences, infections, aging etc. The transformation of normal cells into cancerous ones comprises wide spectrum of biochemical pathways. It is just the complexity of the biology that has hampered the development of effective and specific cancer therapies.

Oral cancer is the sixth most frequent cause of cancer incidence and death worldwide. It is reported that around 3,00,000 new oral cancer cases are diagnosed every year globally (Parkin, 2001). In India, oral cancer is highly prevalent, comprising 80,000 new cases diagnosed annually (ICMR Bulletin, 2001). The habit of tobacco chewing and smoking commonly observed in the population are considered as the major etiologic factors for oral cancer. About 90 percent of cancer risk in young people can be attributed to tobacco usage. Clinical, epidemiological and laboratory studies have shown and confirmed an etiological relationship between prolonged tobacco chewing and oral cancer in India (Nair et al., 2004; Gupta et al., 1987, Jussawala and Despande, 1971). Oral cancer incidence is high in Western India (Davidson, 2001). The hospital based cancer registry (2001) of the Gujarat Cancer and Research Institute has reported that majority of cancers were tobacco related cancers (40.4%). Among all tobacco related cancers, oral cavity is the leading site. Oral carcinogenesis is a multistep and multi path process. The disease is many a times preceded by distinct premalignant stages including hyperplasia, dysplasia, and oral submucous fibrosis/leukoplakia. A premalignant lesion is

morphologically altered tissue in which cancer is more likely to develop than in its apparently normal counterparts.

Oral cancer is an epithelial neoplasia. Most of the early events in the emergence of neoplasia involve multiple genetic and biochemical alterations before clinical manifestation. Although the path of genetic information has universally accepted as being from DNA to protein through translation of mRNA, the flow of such genetic information, in many cases, does not end with protein synthesis. It requires post-translation modifications for functional activation of the proteins (Khersonsky et al., 2003). In the post-genomic era, proteomics plays a significant role in understanding of pathophysiology of cancer, cancer diagnosis and anticancer drug discovery. Glycomics, the unique feature of proteomics, is the study of glycosylation patterns of proteins. It includes evaluation of the structures and functions of all the naturally occurring glycans (Hirabayashi and Kasai, 2000). Glycosylation is one of the most ubiquitous forms of post-translational modifications. The protein-carbohydrate interactions are fundamental aspects in intracellular communication. The multiple functions of glycoproteins in biological systems are paralleled by an enormous microheterogeneity of oligosaccharide structures. The cell surface oligosaccharides mediate many cellular interactions and undergo profound modifications during cell differentiation and neoplastic transformation.

Glycosylation, the biochemical process of putting sugars onto proteins and other molecules, is critically important to many of the signaling pathways involved in turning a normal cell into a cancer cell. Cancer being a cellular disease, changes in cell surface glycoconjugates and enzymes involved in cellular metabolism are of major interest in clinical oncology. Aberrant cell-to-cell recognition, cell adhesion, antigenicity and the invasiveness demonstrated by malignant cells are due to altered cell surface composition compared to normal cells. The process of shedding and increased turnover of surface components in normal cells is a natural physiological phenomenon

accompanying growth and cell proliferation (Black, 1980). Cancer cells continuously shed their surface components into body fluid, which can be used as tumour markers for various malignancies (Bates, 1991). The alterations in glycan levels are the primary molecular phenotypes with functional significance in cancer, rather than secondary effects of changes in the proteins to which they are attached. In cancer cells, the amounts of certain carbohydrates are present in usually high quantities. The core structure of the glycans is rich in mannose, N-acetyl glucosmine, galactose and glucose, which vary from protein to protein. Various biological functions of oligosaccharides are carried out by the terminal elements of the glycans. Sialylation and fucosylation are typical terminal modifications that mediate specific biological functions. Both, sialic acid and fucose contain structural features, which distinguish them from the monosaccharides that make up the common core structures. These terminal epitopes of glycoproteins have been proposed to play a significant role in cell-cell interactions, in the development of cell adhesion and in malignant transformation (Hakomori, 1989). Significant co-relational patterns between altered glycosylation and clinical usefulness have further fueled interest in their potential biological importance in cancer research.

Aberrant sialylation in cancer cells is a characteristic feature associated with malignant properties including invasiveness and metastatic potentials. Cellular sialic acid contents are mainly controlled metabolically by sialyltransferases (SiT) and sialidases. Expressions of SiTs and sialidases are often de-regulated in cancer. Sialic acid is linked through three linkages to glycoproteins catalysed by specific SiTs. Increased SiT activities in serum and tissues of cancer patients are found to be positively associated with tumour burden and invasive properties. It has been documented that SiT inhibitors reduce metastatic potentials of cancer cells. The altered expression of sialidase observed in cancer has suggested its involvement in different malignancies. α-L-fucose occupies terminal position at non-reducing ends of oligosaccharide chains of glycoproteins. It is found as α1,3-, α1,4- or α1,6-linkages and

incorporated by specific fucosyltransferases (FucT). One of the most common changes in glycoconjugates during malignant transformation is fucosylation. The tumour cells modulate its surface to escape recognition by increased fucose levels (Hakomori, 1989). α-L-fucosidase is involved in the breakdown of fucose containing oligosaccharides of glycoconjugates. Alterations in serum levels of α-L-fucosidase have been reported to be useful in cancer diagnosis and management. The Increased expression of several fucosylated antigens is reported to be associated with malignant transformation. The expression of these antigens has been correlated with increased metastatic potentials of tumours and poor prognosis of cancer patients (Noda et al., 2002; Renkonen et al., 1997). However, the studies emphasizing importance of glycoprotein alterations in oral cancer patients as well as in patients with oral precancers (OPC) have not been adequately explored. Therefore, the study of biochemical parameters related to terminal glycosylation changes could be of significant clinical value for oral cancer diagnosis and management.

The most deadly aspect of cancer cell is its ability to spread and metastasize. Cancer cells initially group together to form a primary tumour. Once the tumour is formed, cells may begin to break off from the primary tumour and travel to other parts of the body. Invading cancer cells must breach barriers opposing their movement, which include basement membrane, stromal matrix and cell-cell junctions. The breaking of such barriers requires involvement and interactions of different types of cells, connective tissues and blood vessel components of different organs. The process of tumour progression involves the survival of the fittest cells *in vivo*, and it is rational to mention that these highly selective changes in tumour cell glycosylation are not random or accidental. Furthermore, it is invasion and metastasis that finally kill most patients with cancer, not the simple tumour growth. Thus, due to significant clinical usefulness of glycosylation changes, attention in this research area is indispensable. Hence, many investigators have focused their research efforts on understanding in what ways cancer cells have mutated to circumvent the body's defenses and freely travel to other locations.

Carbohydrate epitopes in tumours mediate tumour cell adhesion through carbohydrate-to-carbohydrate interactions or through interactions of carbohydrate with its binding proteins. Tumour cell adhesion, deformability, motility, cell receptors and expression of cell surface antigens play important role in cancer metastasis. A carbohydrate molecule Sialyl Lewis-X (SLe^X) is frequently expressed on human cancer cells. It serves as ligand for the endothelial cell adhesion molecules and contributes to hematogenous metastasis through the extravasation of cancer cells (Kannagi, 2004). Expression of these carbohydrate determinants is markedly enhanced in cancer cells. However, the molecular mechanisms that cause cancer-associated expression of cell surface antigen, SLe^X have not been well understood. Aberrant expression of cell surface glycoproteins is associated with tumour formation and metastasis. In cancer cells, normal adhesion molecules are found to be missing or are compromised. Cadherins, a family of intercellular adhesion protein molecules, play a major role in keeping cells together and greatly contribute for invasion and metastasis. A subtype of this family, E-cadherin, a 120 kD ($E\text{-}cad^{120}$) transmembrane glycoprotein, is the adhesion molecule found in mammalian cells. Tumour cell dispersion largely relies on the loss of homotypic cell-cell adhesion. The E-cadherin/ catenin complex plays a crucial role in epithelial cell-cell adhesion and in the maintenance of tissue architecture. Modifications in the expression or functions of this complex result in a decrease of adhesive properties of the complex, which in turn convert cell from static to a motile status. The post-translational cleavage of native $E\text{-}cad^{120}$ removes its binding domain and renders soluble form of E-cadherin. Accumulation of this soluble form is found during malignant transformation and promotes cell invasion (Rashid et al., 2001).

In spite of a number of studies aimed at the improvement for early diagnosis and management of patients with oral carcinoma, their prognosis remains poor. Oral cancer cases in India frequently represent with local or regional metastasis. The overall five-year survival rate of oral cancer patients is less

than 50% and has not changed during the past two decades (Nagler, 2002). Only 15% of the patients are diagnosed when the disease is at a localized stage. The patients with advanced disease most often reflect the spread of the disease to local, regional and distant sites and are poorly controlled by combined surgery/irradiation.

Although, the glycomics approach has significant role in cancer, it has not been explored as much as proteins and nucleic acids. Post-translational glycosylation being the important determinant for characterization of complete proteome, currently; the studies of glycans with glycomics approach have drawn attention of many investigators. Thus, the sugars, biology's next sweet spot for the researchers, can be significantly useful to address current clinical problems of oral cancer in India. Therefore, the present study was undertaken to evaluate significance of glycoprotein changes in patients with OPC and oral cancer patients with the following aspects of oral cancer glycobiology:

1. Study of alterations in total sialic acid (TSA), sialoproteins levels and sialyltransferases, sialidase activities.
2. Evaluation of variations in fucose, fucoproteins levels and fucosidase activity.
3. Electrophoretic profile of serum proteins and glycoproteins.
4. Expressions of sialyl Lewis-X and E-cadherin.
5. To understand the mechanisms and clinical significance of glycobiology of oral cancer and to assess role of these parameters in identification of high risk group, early diagnosis, staging, prognostication and treatment monitoring of oral cancer.