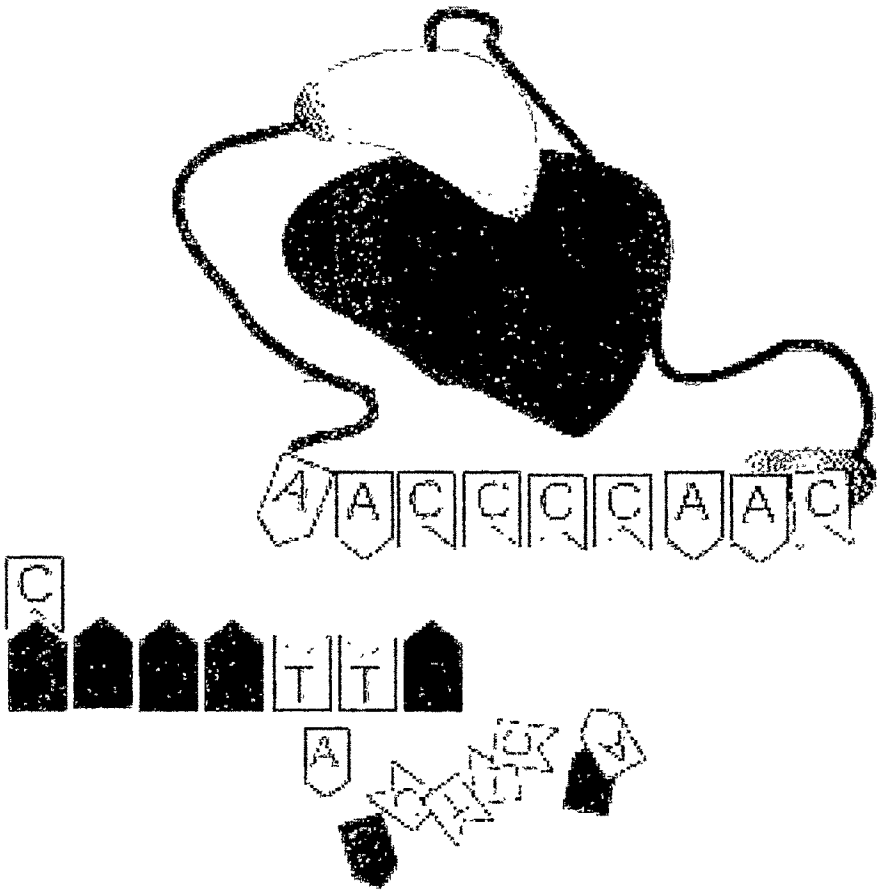


The temptation to form premature theories upon insufficient data is the bane of our profession.....

Sherlock Holmes

Discussion

Cancer is the most dreaded disease, because of the terrifying mystique of relentless growth, invasion and metastasis. It continues to be a major public burden even as we enter the new century. In India, cancer, particularly head and neck cancer, is a major problem. Sincere efforts are being made to develop effective new approaches to control head and neck cancer. Despite advances in therapeutic approaches, prognosis in these patients has remained poor.

Head and neck cancer is a heterogenous disease. Clinical staging systems have been developed to group the patients in categories with regards to their prognosis. Most staging systems group the patients into four groups based primarily on anatomical considerations, with some subdivisions within the various stages (AJCC, 1997, Yorbro et al., 1999). However, even within these subgroups the prognosis varies significantly since these groups comprise a spectrum of patients some of whom can be cured by local therapy while others will have occult metastasis. The presence or absence of lymph node metastasis is considered to be a powerful predictor of survival in cancer patients. However, standard histopathological interpretations routinely underestimate the number of patient with lymph node metastases (Wang et al., 1994). Indeed, routine histopathology examines at the most 1% of the submitted tissue.

Improvements in outcome and cure rates are likely to come from the selection of therapies based on the molecular characterization of the tumors by providing prognostic markers. With a better understanding of tumor biology and the molecular aspects of tumor progression, as well as improved diagnostic techniques, intense efforts are being directed towards identifying and characterizing novel molecular markers for head and neck cancer progression. The ultimate goal of such investigations is to develop reliable prognostic markers that will accurately predict not only the course of an individual head and neck cancer but also the response of that tumor to therapy. Such markers assume a role in the management of patient with head and neck cancer. Therefore, in-depth research in this area is warranted.

Molecular markers are observed to augment clinical and conventional histopathological staging. Molecular probing can detect cancer cells even when they comprise less than 0.01% of the total cell population (Brennan et al., 1995b; Brennan and Sidransky, 1996). The detection of p53 mutations at tumor resection margins heralds future recurrence and has been demonstrated in 38% of tumor-free margins observed by light microscopy. Patients with tumor-free resection margins and also confirmed by p53-staining were found to have no recurrences of the primary tumor. Cervical lymph node metastases have also been

demonstrated by p53 changes, in 21% of histopathologically tumor-free nodes (Brennan et al, 1995b). Molecular probing also examines a larger volume of the tissue, reducing sampling errors present in conventional histopathological examinations. Molecular analysis thus demonstrates a significant clinical implication. To date, a variety of molecular markers have been examined for their ability to predict the disease progression in head and neck cancer patients.

The recognition that tumors have the ability of high proliferation has expanded the body of knowledge on this basic hallmark of malignancy. In recent years, with the advent of molecular techniques, crucial insights into the interplay of various factors at a molecular and genetic level have been gained. A novel postulate that has captured the attention of cancer researchers is that activation of the enzyme telomerase is one of the major pathways by which malignant cells acquire the capacity of high proliferation (Kim et al., 1994). Telomerase has currently captured the attention of cancer researchers all over the world. It is presently evaluated as diagnostic and prognostic marker for cancer. The most important point which has emerged from the studies on telomerase is that it is considered as the universal target for anticancer therapy. To study the clinical implications of these novel molecular markers, this study examined telomerase activation and

telomere length in head and neck cancer. Considerable information is now available about the role of telomeres and telomerase in cancer (Meyerson, 2000, Prescott and Blackburn, 1999). However, there is a dearth of literature on the subject in India, where the studies on head and neck cancer assumes prime importance because of the facts that it is the major malignancy in India (Notani, 1999) and that this malignancy is known to have different etiology in different parts of the world (Paterson et al., 1996). Differences in the etiological factors of head and neck carcinogenesis are known to reflect in molecular characteristics of the tumors. Much research efforts all over the world are being currently focussed on this novel and promising area.

A large body of data now exists to support the hypothesis that the activation of telomerase may be a critical mechanism for tumor progression. With the development of the sensitive TRAP assay for measuring telomerase activity, telomerase expression in numerous malignant, premalignant, and normal tissues were tested (Kim et al., 1994, Shay and Wright, 1996b). Data from these studies showed that telomerase activity is present in almost all tumor-derived cell lines and the majority of malignant tumors but not in most somatic tissues. It was found that 758 of 895 (85%) of malignant tumors, but none of 70 normal somatic tissues, expressed telomerase activity. Numerous studies were

then carried out for validation of these observations. Telomerase activation has been noted in a wide variety of malignancies including breast cancer (Hiyama et al., 1996), head and neck cancer (Mao et al., 1996), prostate cancer (Zhang et al., 1998), lung cancer (Marchetti et al., 1999), thyroid carcinoma (Aogi et al., 1999), colorectal cancer (Yan et al., 1999), brain tumors (Sano et al., 1996), ovarian cancer (Datar et al., 1999), leukaemia (Shay and Wright, 1996a) and skin cancer (Parris et al., 1999). There are substantial but varied reports available on telomerase activation in head and neck cancer (**Table-24**). Califano et al. (1996) have observed telomerase activation in 80% of the head and neck squamous cell carcinoma tissues. Mutirangura et al. (1996) have reported 14 out of 16 head and neck squamous cell tissues to be telomerase positive. Mao et al. (1996) found that all the cell lines derived from head and neck squamous cell carcinoma and 26 of 29 invasive tumors of head and neck showed telomerase activation. Sumida et al. (1999) observed telomerase activation in 25 of 26 malignant lesions of head and neck, while Miyoshi et al. (1999) observed telomerase activation in 30 of 31 malignant oral tumors. On the other hand, Liao et al. (2000) documented telomerase activation in 32 of 39 (82.1%) of oral squamous cell carcinoma tissues. In line with other studies, present study reported telomerase activation in high number i.e. 68.2% of the head and neck cancer tissue specimens.

Table-24

Results from different studies on telomerase activation in head and neck cancer

Authors	Telomerase Activation (Number positive / Number tested)		
	Malignant tissues	*Precancerous lesions*	*Adjacent normal tissues*
Califano et al. 1996	26/35	3/6	NI
Mutirangura et al. 1996	14/16	10/26	NI
Mao et al. 1996	26/29	NI	0/17
Miyoshi et al. 1999	30/31	4/11	0/40
Liao et al. 2000	32/39	12/22	5/13
Sumida et al. 1999	25/26	9/22	0/19

NI: Not Included.

Table-25

Results on telomerase activation in head and neck cancer in Indian population

Authors	Telomerase Activation (Number positive / Number tested)	
	Malignant tissues	*Adjacent normal tissues*
Kannan et al. 1997	75%	Positive
Sharma et al. 1999	100%	63.6%
Patel et al. 1999	78.1%	73.3%

It is noteworthy that, even though there is a flood of reports on telomerase activation in a variety of cancers, from different parts of the world, the data on telomerase activation in head and neck cancer in Indian population is limited. Till date only three reports on telomerase activation in head and neck cancer in Indian population have been documented (**Table-25**). One of them is the report of our preliminary study on telomerase activation in head and neck cancer (Patel et al., 1999). Remaining studies conducted in Indian population, include a

collaborative work carried out by Kannan et al. (1997) who reported telomerase activation in 75% of the malignant tumors of oral mucosa. While the study reported by Sharma et al. (1999) found telomerase activation in 100% of squamous cell carcinomas of aerodigestive tract.

However, in contrast to other reports, we have observed a high frequency of telomerase activation in precancerous/benign tissue specimens as well as in the adjacent normal tissue specimens. Considerable number of studies have examined telomerase activation. However, many studies have not reported the telomerase activation status in adjacent normal and precancerous tissue specimens. A few reports have suggested that telomerase activity is selectively expressed in malignant and germ line cells but not in normal somatic cells (Kim et al., 1994). However, subsequent studies have revealed a different trend. Telomerase activation has been observed in a variety of highly proliferative normal tissues including hematopoietic cells (Buchkovich and Greider, 1996) and oral mucosa (Sharma et al., 1999). Bachor et al. (1999) interestingly reported that telomerase activity was expressed in all normal epithelia as well as in all precancerous lesions. Also, adenocarcinomas showed high telomerase activity in a study on various tissue of the gastrointestinal tract. Liao et al. (2000) found telomerase activation in 54.5% of leukoplakia samples and 38.5% of normal oral

mucosa samples. Mao et al. (1996) documented telomerase activation in all dysplastic and hyperplastic head and neck lesions and 38.7% of adjacent normal tissues. Sumida et al. (1999) reported telomerase activity in 49% of precancerous tissues and none of adjacent normal oral mucosa. Among the studies in Indian population, Kannan et al. (1997) have observed the adjacent normal mucosa to be positive for telomerase activation. While, Sharma et al. (1999) reported telomerase activation in 14 of 22 adjacent normal tissues. The present study has also found higher frequency of telomerase activation (52 out of 103) in adjacent normal tissues. The higher frequency of telomerase activation in adjacent normal tissues in the Indian population may be possibly due to the differences in the etiological factors. Tobacco is considered to be the major causative factor for head and neck cancer. Chronic carcinogenic insults in the form of tobacco, which affects multiple cells, may result in the process of field cancerization where multiple independent malignant and premalignant foci develop (Slaughter et al., 1953). Certain genetic and biochemical changes might be taking place in certain mucosal cells, even though these cells may yet be histopathologically normal. The evaluation of surgical margins is usually made by histopathological assessment of resected tumor specimens. However, local recurrence may occur even with histopathologically negative surgical margins (Vikram, 1984). Mutated clones have also been reported even in

histopathologically normal epithelium (Larson et al., 1998). Several mutations at potential sites including tumor-suppressor genes, indicating that genetic abnormalities accumulate before histopathological changes, were detected. Therefore, it is possible that in the patients showing telomerase activation in adjacent normal tissues, the apparently adjacent normal tissue may have undergone certain genetic and biochemical changes or certain malignant cells might have spread to the adjacent normal area from the tumor site. This may suggest the presence of occult malignant disease even after surgical resection of the tumor.

The general criteria used at present to evaluate the disease and its progression, are clinical and histopathological characteristics. However, as discussed earlier, this system of analysis is still lacking in many aspects, and shows heterogeneity. Telomerase activation has been compared with the clinicopathological characteristics in different malignancies, and varying results have been reported. Wisman et al. (2001) noted that telomerase was not applicable as a prognostic factor in early stage cervical cancer patients. Hoos et al. (1998) observed significant correlation between enzyme activity and tumor size, lymph node status and stage of the disease in breast cancer patients. On the other hand, Bednarek et al. (1997) did not find any significant

correlation between telomerase levels and lymph node status or tumor size in breast cancer patients. Mokbel et al. (2000) have reported that telomerase reactivation is significantly associated with lymphovascular invasion in breast cancer and may reflect the metastatic potential of the disease. They also reported that telomerase activity was significantly associated with nodal metastases but not with tumor grade or tumor size. Miyoshi et al. (1999) also could not establish any association of telomerase activation with grade or stage of oral cancer. In the present study, we carried out multivariate analysis to evaluate any possible correlation between telomerase activation and the clinical and histopathological characteristics. No statistically significant correlation between telomerase activation and any of the clinicopathological parameters including age, gender, anatomic site, nodal involvement, histologic grade and nuclear grade in patients with head and neck cancer could be established. However, we did find some striking features in the analysis. Adjacent normal tissues in patients with advanced stage of disease, particularly stage IV disease, showed highest frequency of telomerase activation. This observation may suggest the possibility of spread of the tumor in the adjacent area. Also, patients with poorly differentiated tumors and patients with nuclear grade III tumors showed the highest frequency of telomerase activation in both adjacent

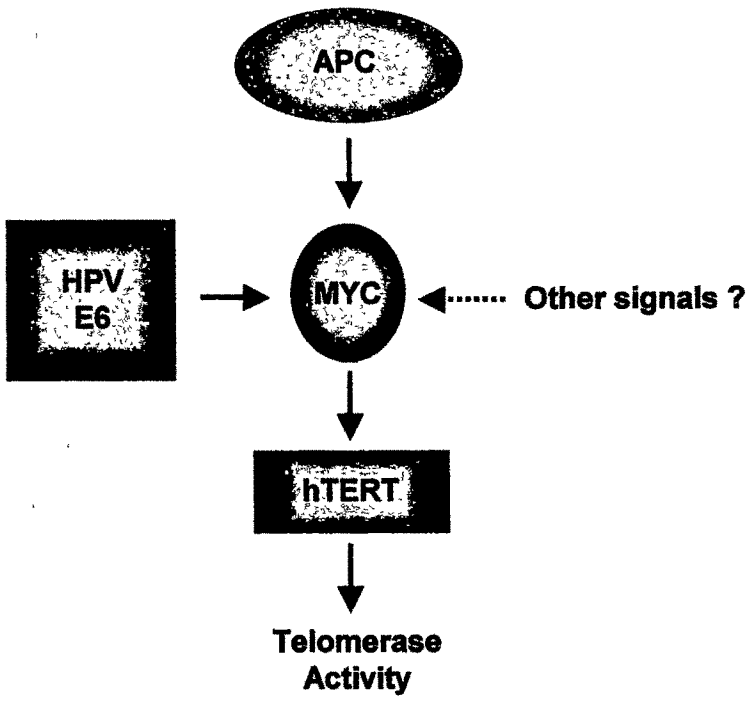
normal and malignant tissues. This observation suggests trend of higher telomerase activation with increasing grade of the disease.

Recent advances in identifying the components and genes encoding them have improved the understanding of telomerase activation (Greider, 1999; O'Rielly, et al., 1999; Weilbaecher and Lundblad 1999). Telomerase is a ribonucleoprotein enzyme composed of a catalytic protein subunit (hTERT) and stably associated RNA moiety (hTR). Telomerase-associated protein 1 (hTP1) is also known to be associated with telomerase activity. There is compelling evidence supporting that the regulation of telomerase is executed primarily through the transcriptional control of hTERT (Meyerson et al., 1997; Nakamura et al., 1997; Nakano et al., 1998). Takahashi et al. (2000) have observed that the expression level of hTERT mRNA correlated well with relative telomerase activity. While, hTR was detected in all tissues with higher expression levels in hepatocellular carcinoma tissues. Thus, telomerase activation during hepatocarcinogenesis might be regulated only by hTERT and that increase in telomerase activity level might be regulated by both hTERT and hTR. Poremba et al. (2000) have demonstrated significant correlation between telomerase activity levels and expression levels of hTERT and hTR in neuroblastoma. Harada et al. (1999) have shown that both hTERT and hTR are essential for telomerase activity in malignant lymphoma of central nervous system. These telomerase

components have also been studied in human oral lesions (Fujimoto et al., 2001; Sumida et al., 1999). The studies documented that hTR and hTP1 mRNA expression was detected in all malignant and normal oral tissues with or without telomerase activity while all lesions expressing hTERT were telomerase positive (Sumida et al., 1999). The central fact emerging from various studies is that all the three components hTERT, hTR and hTP1 are essential for telomerase activity; however, hTERT is the controlling factor for telomerase activation. Expression of hTERT determines telomerase activity and the level of expression of hTERT and hTR determines the level of telomerase activity. Recent evidence suggest that expression of hTERT is reported to be triggered by human papilloma virus (HPV) E6 oncogene and MYC (**Figure-19**). Activation of telomerase by E6 occurs, at least in part, through activation of the MYC oncogene. Expression of the MYC oncogene in primary human epithelial cells that lack telomerase activity leads to telomerase activation by up-regulating the mRNA encoding hTERT (Wang et al., 1998). Futhermore, the recent demonstration that MYC is the target for APC provides evidence for another pathway by which telomerase may be up-regulated (He et al., 1998). Telomerase is also observed to be regulated at protein level, by nuclear translocation and phosphorylation (Liu, et al., 2001).

Figure-19

Telomerase activation pathways



The phosphorylation is shown to be brought about protein kinase C-zeta (Yu et al., 2001). TGF-β, Rb and E2F-1 are also known to regulate telomerase activity (Crowe and Nguyen, 2001; Yang et al., 2001)

Current advances in biochemical and genetic experiments have established a clear link between telomere maintenance and the process of immortalization, chromosomal stability and tumorigenesis (Colgin and Reddel, 1999, Oulton and Harrington, 2000, Artandi and DePinho,

2000). Telomerase attrition at every cell division is postulated to limit the lifespan of human cells, while activation of telomerase is proposed to be an essential step in cancer cell immortalization and cancer progression (Meyerson et al., 2000). It is believed that telomeres are stabilized by telomerase at a very short length. Telomere length is generally measured as the size of terminal restriction fragments. They appear as a smear on the gel, as the number of telomeric repeats vary on different chromosome ends (Autexier and Greider, 1996). The present study analysed telomere length changes in head and neck cancer patients by measuring TRF length in adjacent normal and malignant tissue specimens. It was evident from the study that the mean peak TRF length in malignant tissue (8.23 kb) was significantly lower as compared to that in adjacent normal tissues (10.14 kb). Numerous studies have been conducted to elucidate the changes in telomere length in various cancers. Hiraga et al. (1998) have documented reduced TRF length in majority (14 of 15) of high grade brain tumors. Kammori et al. (2000) found mean telomere length to be 9.1 kb in human thyroid cancer tissues which was significantly lower than that observed in adjacent normal tissues (12.9 kb). In a study on hepatocellular carcinoma, Ohashi et al. (1996) reported mean TRF length to be 5.6 kb in malignant tissues, which was significantly lower than that in corresponding noncancerous liver tissues with viral infection (9.0 kb) and viral free

control tissues (12.4 kb). Ohyashiki et al. (1994) found that telomere shortening was associated with disease evolution patterns in myelodysplastic syndromes. Engelhardt et al. (1997) reported that TRF in colon tumors, with the peak value of 4.8 kb, was significantly shorter than those of the adjacent normal tissues. Huang et al. (1998) interestingly documented that out of 28 cases of human hepatocellular carcinoma analysed, telomere length was shortened in 11 cases, lengthened in 6 cases and unaltered in 11 cases as compared with non-tumor tissues. Imam et al. (1997) suggested that stable telomeric repeat lengths may be a molecular phenotype of the early stages in progression of breast cancer. After analysing tissue specimens from 35 different tumors and adjacent normal tissues, Schmitt et al. (1994) concluded that there was no general tendency to telomere reduction in malignant tissues. Chen et al. (2000) have documented mean telomere length to be 11.9 kb in intracranial meningiomas. They also observed elongated telomeres in 6 out of 13 patients with malignant or atypical meningiomas and elongation of telomeres implicated high potential for malignant behaviour in these tumors. Taken together, earlier studies have shown that there is great variation in telomere length in different types of cancers. There are numerous reports available on telomere length in different types of cancers; however there are no reports on head and neck cancer. To the best of our knowledge, the present study is

the first report on detailed analysis of telomere length in head and neck cancer. It was observed that the mean peak TRF length in adjacent normal and malignant tissues was 10.14 kb and 8.23 kb, respectively. The mean peak TRF length was significantly lower in the malignant tissues as compared to the adjacent normal tissues. Paired "t" test analyses the difference between the values in adjacent normal and malignant tissue from the same patient. This analysis also revealed the same trend that even when individual patients were considered, malignant tissues showed significantly shorter peak TRF length as compared to the adjacent normal tissues. Indeed, more than 90% of the head and neck cancer patients showed shorter peak TRF length in malignant tissues.

Clinical presentations and histopathological features are the main criteria currently used for prognostication of cancer patients. Therefore, the correlation between telomere length and clinicopathological characteristics in the head and neck cancer patients was assessed. However, no correlation could be observed between peak TRF length and clinicopathological characteristics including age, gender, anatomic site, stage, nodal status, histologic grade and nuclear grade. Once again, there are controversial reports available on these aspects. Huang et al. (1998) have observed that telomere length does not correlate with any

clinical parameters including tumor size, Edmondson's grade or recurrence of hepatocellular carcinoma. Engelhardt et al. (1997) found telomeres to be 0.6 kb longer in late stage cancers (Dukes C+D) as compared to that in early stage cancer (Dukes A+B). Ohyashiki et al. (1994) noted that telomere shortening at the time of diagnosis of myelodysplastic syndrome might indicate a poor prognosis. Ohashi et al. (1996) documented that reduction in TRF length in tissues of hepatocellular carcinoma increased with the tumor diameter, although this failed to attain statistical significance. Hiraga et al. (1998) observed that shortened telomeres correlated with the aggressive growth of high-grade neuroepithelial tumors. In contrast, Chen et al. (2000) reported that elongation of telomere length implicated the high potential for malignant behaviour in malignant meningiomas. In the present study, telomere length emerged as an independent factor, showing no correlation with the clinical and histopathological features in head and neck cancer. However, in most of the groups of patients grouped on the basis of stage of the disease, nodal involvement, histologic grade of the disease or nuclear grade of the disease, the peak TRF length was found to be significantly lower in malignant tissues as compared to that in adjacent normal tissues.

The measurements of telomere length have been variable and the reason for this could be the true biological variation in telomere repeat number between chromosomes. Another reason may be the fact that southern blot measurements rely on measuring a terminal restriction fragment which also includes a subtelomeric region which itself varies between chromosomes (Autexier and Greider, 1996). The dynamics of telomere length depends on the balance between the mechanisms that shorten the telomere length e.g. cell proliferation and mechanisms of telomere extension e.g. telomerase. Telomerase activation is the most widely used mechanism for stabilization and elongation of telomeres (Meyerson, 2000). In the present study we observed that there was no correlation between telomerase activation status and telomere length. Studies on telomerase-positive immortalized cells (Bryan et al., 1998, Jones et al., 1998) and telomerase-negative normal cells (Martens et al., 1998) reveal evidence for the existence of factors other than telomerase that regulate telomere length. A number of proteins are being reported to bind to the telomere and/or affect telomere length. These proteins may affect telomere maintenance by modulating the activity of telomerase, or by protecting chromosome ends against loss of telomere repeats. TRF1, the fisrt reported mammalian telomere binding protein identified by Zhong et al. (1992), mediates parallel pairing of telomeric tracts (Griffith et al., 1998) and is likely to have a role in telomere

protection and may be important for regulating telomere length through inhibition of telomerase (van Steensel and de Lange, 1997). TRF2, a distantly related homolog of TRF1, also binds double-stranded telomere repeats and is said to have an important role in telomere protection (Broccoli et al., 1997). Overexpression of dominant negative alleles of TRF2 in human cells caused loss of the terminal single-stranded 3' overhang, an increase in chromosome end-to-end fusions, and irreversible growth arrest in a senescence-like state (van Steensel et al., 1998). Tankyrase, the enzyme which co-localizes with TRF1 at telomeres is also seen to have an important role (Smith et al., 1998). A recently identified putative telomere-binding protein, UP1, is proposed to play a role in recruiting telomerase to the ends of telomeres. UP1 is the amino-terminal fragment of a heterogeneous nuclear ribonucleoprotein A1 (LaBranche et al., 1998). UP1 appears to bind both telomeric repeats and telomerase. Therefore, it is proposed that proteolytic processing of A1 to UP1 may recruit telomerase to the ends of telomeres. It has been shown that in some cases, cells that escape from crisis have no detectable telomerase activity (Bryan et al., 1995). Southern blot analysis with a telomere-specific probe showed that telomerase-negative immortalized cells have a characteristic pattern of TRF length that ranges from very short to abnormally long (Bryan et al., 1997). In clonally derived cell lines immortalized *in vitro* it was

observed that the acquisition of abnormally long telomeres coincided with escape from crisis, demonstrating the existence of one or more Alternative Lengthening of Telomeres (ALT) mechanisms (Bryan et al., 1995). Human fibroblasts and other cells of mesenchymal origin seem more likely to utilize the ALT pathway for immortalization than epithelial cells, although a minority of *in vitro* immortalized epithelial cell lines, carcinoma-derived cell lines, and carcinomas do exhibit ALT (Bryan et al., 1995, 1997). The ALT mechanisms might involve recombination as documented for yeast (Kass-Eisler and Greider, 2000). Chen et al. (2001) observed that either type I or type II recombination pathways can allow cells to survive in the absence of telomerase and that elimination of both pathways in a telomerase mutant leads to the inability to elongate telomeres and ultimately cell death. Thus, there are more than one factors and their interplay, on which telomere length depends.

The clinical implications of understanding the invasive and metastatic proclivities of an individual tumor are substantial. Upon diagnosis, the critical questions that clinicians face are: what is the most effective treatment, and what are the side effects? The use of systemic therapy in patients with seemingly localized disease needs to be guided by the likelihood of occult metastasis, as well as by knowing when the occult

metastasis will become overt. To design a treatment plan, these characteristics must be ascertained for individual patient. A thorough understanding of the clinical and molecular characteristics of tumor may aid in this determination and allow the fashioning of a specific therapy. The two year disease free survival analysis in this study showed noteworthy results. The patients showing telomerase activation in adjacent normal tissue and patients showing higher peak TRF length in malignant tissues had poor prognosis. Thus, the telomerase activation in adjacent normal tissue may be suggestive of residual or occult metastatic disease after incomplete surgical removal of the tumor. Similarly higher peak TRF length in malignant tissue may represent higher proliferative capacity of the malignant cells leading to bad prognosis. Therefore, the patients showing telomerase activation and or higher peak TRF length may be in need of a close follow-up and aggressive anticancer therapy. Thus, telomerase activation and telomere length have the potential to discern the patients who needed aggressive therapy and spare those without occult metastatic disease. Telomerase activation has also been investigated as a prognostic marker in a variety of cancers and of telomerase activation is reported to be an important prognosticator. Progression of head and neck cancer was observed to be accompanied by a parallel and continuous increase in telomerase activity by Soria et al. (2001). High telomerase activation

has been shown to be an independent prognostic indicator of poor outcome in colorectal cancer (Tatsumoto et al., 2000). Marchetti et al. (1999) have documented significant association between telomerase activity and both disease free and overall survival in patients with stage I non-small cell lung carcinoma. Summarising this, the analysis of telomerase and telomere length can help the clinicians in discerning patients in need of a close follow-up and tailoring their treatment.

In a nutshell, the present study implicates clinical usefulness of telomerase activation and telomere length in head and neck cancer patients. There were some significant differences observed in the pattern of telomerase activation in the Indian population. A surprisingly high number of adjacent normal tissue specimens were found to show telomerase activation, which may be due to the field cancerization effect following chronic carcinogenic insults to the mucosa, mainly in the form of tobacco. Both telomerase activation and telomere length emerged as independent featurers in head and neck cancer. These parameters were also found to be useful prognostic markers, identifying the patients in need of close follow-up and aggressive therapy. Thus, identification and characterization of molecular markers further enhance the ability of clinical and histopathological features in diagnosis, prognostication and management of cancer patients. Understanding the biological pathways

which regulate the molecular signature of the disease offers a great promise in therapeutic outcome of the patients. Such studies will help in furthering our understanding of the contributions of molecular markers in head and neck carcinogenesis and enable us to fully exploit their potential clinical implications. Management of head and neck cancer patients may therefore become more rational and conservative. These advances, which are just beginning to be realized, will herald a sustained reduction in morbidity and mortality rates in head and neck cancer patients.