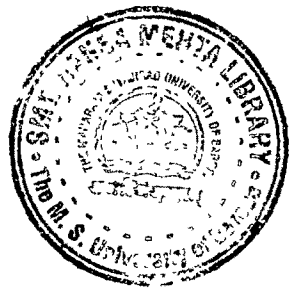

SUMMARY OF THE THESIS ENTITLED

EVALUATION OF BIOCHEMICAL CHANGES IN CANCER

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SUMMARY

Cancer has emerged as a mammoth medical problem. In India, head and neck cancer is particularly an alarming problem, with an incidence as high as 40% of all cancers. The preponderence of head and neck cancer can be considered to be due to environmental factors. Tobacco usage has been associated with cancer since a long time. In Indian population, habit of tobacco consumption has been observed at a high frequency and in a variety of modes, which are more harmful than mere cigarette smoking observed in the western countries. These differences in the etiology of the disease are known to reflect as changes in the molecular characteristics of the tumor. Furthermore, molecular analyses of the disease have spawned new insights into diagnostic, prognostic and therapeutic management of cancer. Therefore, it is important to study the molecular changes in head and neck cancer in Indian population.

One of the most compelling hypotheses investigated in recent years is the telomere-telomerase hypothesis, which implicates telomere attrition and telomerase activation as critical factors in cancer. Telomeres and telomerase have, at present, gained utmost attention of the researchers. In-depth studies are being carried out to elucidate effective pathways in exploiting telomerase for early detection, prognostication and treatment

of cancer. The present study investigated telomere length and telomerase activation in head and neck cancer patients who visited the out patient's department of The Gujarat Cancer & Research Institute, Ahmedabad. One hundred and ten patients with head and neck cancer and 40 patients with precancerous conditions were enrolled for the study. Detail clinical history was obtained from each patient, which included details regarding age, gender, anatomic site of the lesion and habit of tobacco consumption. All the patients were followed-up and details regarding treatment given, operative findings, stage of the disease, histologic grade of the disease, nuclear grade of the tumor, and nodal involvement were collected. During the follow-up, details regarding disease status, clinical appearance of recurrence/metastasis and disease free survival as well as overall survival time were also recorded. Telomerase activation was evaluated using Telomeric Repeat Amplification Protocol (TRAP), which gave a characteristic 6 base pair DNA ladder. While telomere length was measured using southern hybridization with digoxigenin labeled telomeric probe $(TTAGGG)_4$. Telomere length was measured in the form of peak Terminal Restriction Fragment (TRF) length in each specimen. Any possible correlation of these markers with the clinical and histopathological characteristics were analysed using multivariate analysis. Kaplan and Meier survival analysis was carried out to establish the association between the

parameters and disease free survival in head and neck cancer patients. The striking observations of the study are summarised below:

- Telomerase was observed to be activated in most i.e. 75 of 110 (68.2%) malignant tissue specimens from the head and neck cancer patients.
- Analysis of tissues from patients with precancerous lesions/conditions showed that telomerase activation was present in 35 of 40 (87.5%) of the tissue specimens.
- Telomerase activation was observed in a surprisingly high number i.e. 52 of 103 (50.5%) of the adjacent normal tissues from head and neck cancer patients.
- Telomere length analysis in patients with head and neck cancer showed that mean peak TRF length in adjacent normal and malignant tissues was 10.14 kb and 8.23 kb, respectively. Student's "t" test analysis showed that the mean peak TRF length in malignant tissues was significantly shorter as compared to that in adjacent normal tissues. The paired "t" test analysis, which analysed the difference between the adjacent normal and malignant tissues from each patient individually, also showed the same trend i.e. malignant tissue specimens had shorter peak TRF length. Also, more than 90% of the head and neck cancer patients showed shorter peak TRF

length in malignant tissues as compared to the adjacent normal tissues.

- Multivariate analysis to establish correlation between telomerase activation and clinicopathological characteristics revealed that there was no significant correlation between telomerase activation and age, gender, anatomic site of the disease, stage of the disease, nodal involvement, histologic grade of the disease or nuclear grade of the disease in patients with head and neck cancer. However, adjacent normal tissues in patients with disease of advanced stage, particularly stage IV disease, showed highest frequency of telomerase activation. 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.
- Similarly, multivariate analysis for peak TRF length and clinicopathological characteristics revealed that there was no correlation between peak TRF length and age, gender, anatomic site of the disease, stage of the disease, nodal involvement, histologic grade of the disease or nuclear grade of the disease in patients with head and neck cancer. However, in most of the groups considered 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.

- Spearman's correlation analysis revealed that telomerase activation and peak TRF length were independent of each other.
- Kaplan and Meier analysis for two year disease free survival revealed that head and neck cancer patients who showed telomerase activation in adjacent normal tissues had significantly poor two year disease free survival as compared to the patients who did not show telomerase activation in adjacent normal tissues.
- Kaplan and Meier analysis for two year disease free survival revealed that head and neck cancer patients who showed peak TRF length higher than the median value in malignant tissues, had significantly poor two year disease free survival as compared to the patients showing peak TRF length lower than the median value in malignant tissues.

CONCLUSION

In conclusion, these observations revealed that telomerase activation was observed in most of the head and neck cancer patients and telomere length was significantly shorter in malignant tissue specimens. These parameters can serve as useful adjunct to the current battery of diagnostic tools. In contrast to reports from other countries, telomerase

activation was observed in high number of adjacent normal tissues, which could be regarded as the field cancerization effect induced by tobacco. It may predict presence of malignant or transformed cells in the apparently normal tissues adjacent to the tumor. Indeed, telomerase activation in adjacent normal tissues predicted a poor two year disease free survival. Similarly, longer peak TRF length in malignant tissues also predicted poor disease free survival, suggesting that malignant cells with longer telomeres had greater ability towards disease progression. These patients may be considered for close follow-up and aggressive anticancer therapy. Thus, findings of the current study can provide a molecular basis of refining the treatment protocols. Analysis of telomerase activation and telomere length can help clinicians in diagnosing the disease and also discerning the patients at risk of disease progression. Hence, these parameters might aid in establishing an individually tailored anticancer therapy. Taken together, findings of the study implicate the clinical usefulness of these parameters in management of head and neck cancer. Insights generated from this investigation will undoubtedly lead to improved strategies for management of cancer patients.

CONCLUDING REMARKS

During the last couple of decades we have witnessed the burgeoning of molecular study of cancer as a major field of cancer research. Together with the past accomplishments, emerging details from the present study form a remarkable picture of progress in understanding and application of molecular markers in cancer. It is, however, important not to lose sight of the fact that the process of telomere maintenance, either by telomerase activation or alternate mechanisms is complex. Clearly, the current study and other reports till date have revealed the tips of these mechanisms and much work still lies ahead. The outstanding questions that still need to be thoroughly answered are:

(i) What is the role of telomerase components (hTR, hTERT and hTLP1) in cancer and how can these components be exploited for the better management of cancer patients? It is known that all the three components are essential for telomerase activity, however, hTERT is the determining factor for telomerase activation. The mechanisms inducing hTERT expression are still under study.

(ii) What is the detailed pathway of telomerase activation? As yet, only two pathways are known: telomerase activation by HPV E6 and MYC.

(iii) What are the pathways of telomere maintenance other than telomerase activation? Alternate mechanisms for telomere extension have been well reported but poorly characterized.

(iv) What are the factors influencing telomere length? Many factors including TRF 1 and 2, and UP1 have been identified, but the detailed pathways remain to be elucidated.

(v) How can telomerase activation be blocked? Many telomerase inhibitors have been proposed, however, they are still at the laboratory level, and the challenge lies in application of current knowledge in the subject towards treatment of this life-threatening disease.

Such analyses will further improve our understanding of the biological pathways regulating the molecular signature of the disease, offering great potential for better management of cancer patients. This will create a realistic and imminent promise to achieve victory in the fight against cancer.