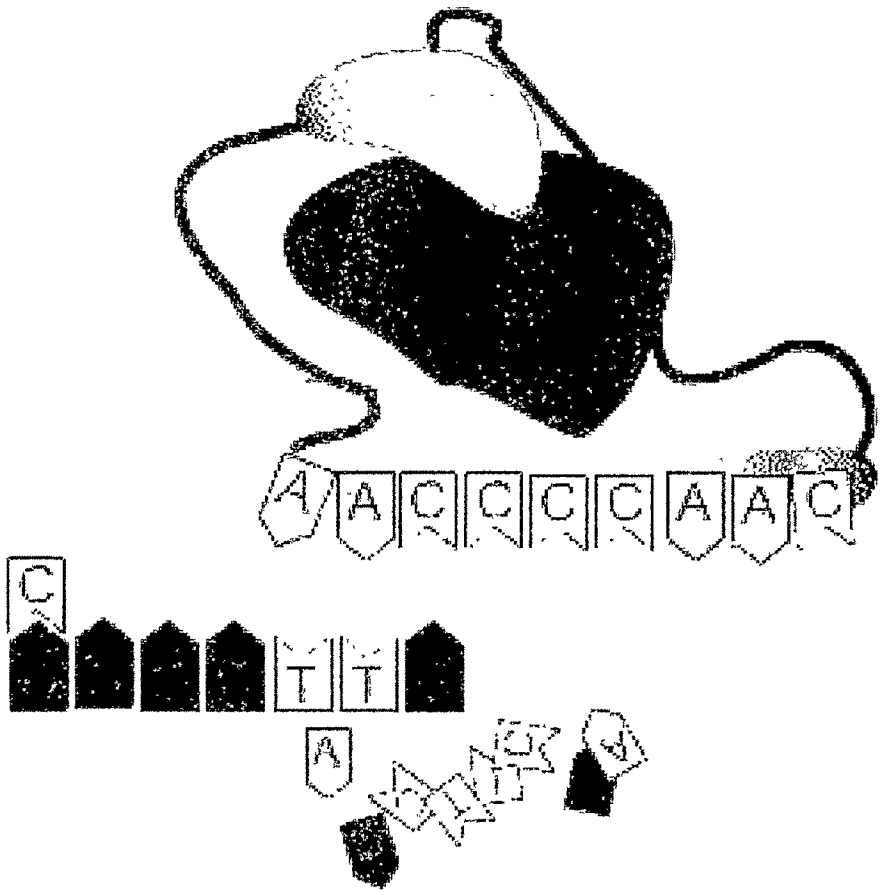


*To steal ideas from one person is plagiarism, to steal ideas from many is research... ...*

***Anonymous***

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# Evaluation of telomerase activation in head and neck cancer

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carried out on head and neck cancer [13–15] To the best of our knowledge there is only one report on telomerase activation from India to-date [8]. The etiology of tobacco related cancers in Indian population is different from that in Western countries [16]. Tobacco chewing, snuffing, bidi smoking, reverse smoking, etc. are common in Indian population in contrast to Western population where cigarette smoking is more prevalent These differences in the etiological factors may reflect in molecular changes in tumour characteristics [16] It has been reported that tumours in Indian population show involvement of *ras* oncogenes which is uncommon in Western countries [16]. Hence, it is necessary to study the role of telomerase activation in head and neck cancer in India. In the present study we evaluated telomerase activation in tumour tissues from head and neck cancer patients, tissues from precancerous/benign lesions and adjacent normal tissues

## 2. Materials and methods

### *2 1 Patients and tissue samples*

Forty-two patients referred to The Gujarat Cancer and Research Institute, Ahmedabad as suspected cases of head and neck lesions or tumours were included in the study after obtaining due consent. Tumour tissue samples from these patients were collected either during surgical biopsy or surgical resection of the tumours It was confirmed that none of the patients received any treatment for the disease before sample collection for the study, with exception of one patient with recurrent leukoplakia. The histopathological reports revealed that 35 patients had head and neck cancer and 7 patients had oral precancerous/benign diseases Adjacent normal tissues were also collected from most of these patients Four patients with head and neck cancer also had precancerous lesions In one case, we could collect both precancerous and malignant tissue samples from opposite cheeks. In the other three cases, tissues could be obtained only from precancerous/benign lesions Detailed clinical and histopathological characteristics of these patients are provided in Table 1 Histopathological classification was done according to the TNM classcation system (UICC) Precancerous/benign lesions included oral submucous fibrosis ($n = 4$), oral leukoplakia ($n = 3$), haemangioma ($n = 3$) and acanthosis ($n = 1$). The tissue samples were snap frozen in liquid nitrogen and stored at −80°C until analysis

### *2 2 Telomerase assay*

Telomerase activity was assayed using the telomeric repeat amplification protocol (TRAP) assay [3,17]. Telomerase-PCR-ELISA kit (Boehringer Mannheim, Germany) was used to perform TRAP assay Manufacturer's instructions were followed with necessary modifications Frozen tissue samples were washed with Tris buffered saline pH 8, crushed in liquid nitrogen, suspended in 200 µl lysis buffer and incubated on ice for 30 min. The lysates were then centrifuged at 16 000 *g* for 20 min at 4°C The supernatant was stored in aliquots at −80°C until analysis Protein concentration of these tissue extracts was measured by Lowry's method [18]

For TRAP assay, tissue extract equivalent to 50 µg protein was added to 25 µl of reaction mixture (containing biotin labeled primers) and incubated at 25°C for 30 min for telomerase mediated extension of primer. Telomerase activity was stopped by heating at 94°C for 5 min. Thirty-one PCR cycles were carried out subsequently The amplification product was electrophoresed on 15% denaturing polyacrylamide gel. This was followed by its electrotransfer on nylon membrane. The amplification product was then detected using Biotin Luminescent Detection kit (Boehringer Mannheim, Germany). Briefly, the membrane was incubated in blocking solution followed by streptavidin-alkaline phosphatase solution. The membrane was then washed and Disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2′-(5′-chloro)tricyclo[3.3.1.1 $^{3,7}$]decan}-4-yl)phenyl phosphate (CSPD) was evenly spreaded on it After a brief incubation at 37°C for 10 min, the membrane was exposed to X-ray film for luminescence detection Positive control cell extract was provided by the company alongwith the kit For negative control, lysis buffer was used instead of cell extract and proceeded similarly

## 3. Results

Samples producing a characteristic 6-basepair DNA ladder were considered to be telomerase positive. 6-basepair DNA ladder was observed in positive controls whereas the ladder was absent in negative controls Fig 1 shows telomerase activity in malignant, precancerous and adjacent normal tissue samples and controls Lanes 1 and 2 represent telomerase negative adjacent normal tissue sample and telomerase positive malignant tissue sample respectively, from a patient with carcinoma of larynx Lanes 3 and 4 represent telomerase positive adjacent normal tissue sample and malignant tissue sample, respectively, from a patient with carcinoma of buccal mucosa Lanes 5 and 6 represent positive and negative controls, respectively Lane 7 represents a precancerous (oral submucous fibrosis) tissue sample from a patient who presented with carcinoma of buccal mucosa at the time of diagnosis Lane 8 represents precancerous (oral leukoplakia) tissue sample from a patient who had no evidence of carcinoma Clinical details of all the patients included in the study and result of TRAP assay are provided in Table 1 Table 2 shows frequency of

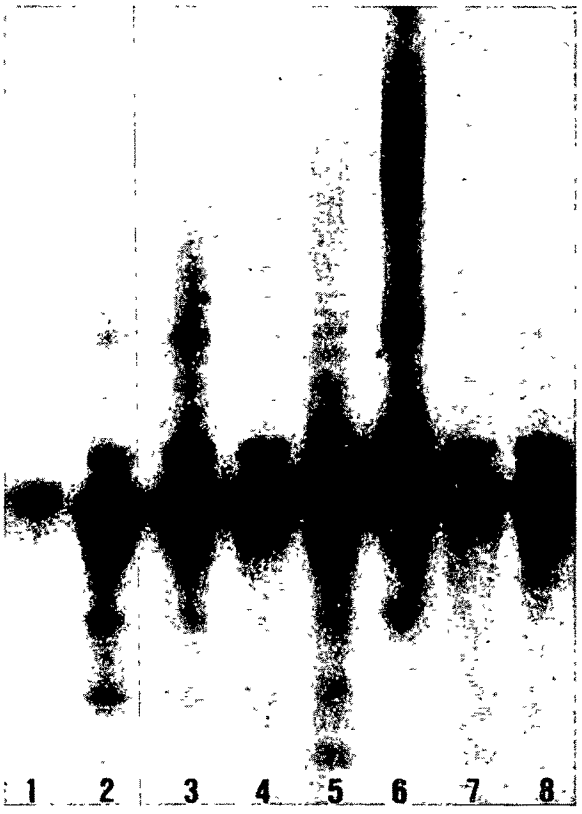


Fig 1 Telomerase activity in malignant, precancerous and adjacent normal tissue samples as well as positive and negative controls

Table 2
Telomerase activity in malignant, adjacent normal and precancerous tissues

| | Telomerase activity | |
|---|---|---|
| | Positive | Negative |
| Head and neck carcinoma tissues (32) | 25 | 7 |
| Precancerous/benign tissues (11) | 11 | 0 |
| Adjacent normal tissues (30) | 22 | 8 |

It is believed that interference with telomerase activity could represent a universal and highly effective approach to cancer therapy [19] The specific association of human telomerase activation with immortal cancer cells has been well documented [4–8,20] Until recently telomerase expression in normal cells was believed to be restricted to germ cells, activated β lymphocytes and rare stem cells [20] However, subsequent reports have altered this belief Many recent reports have suggested telomerase activation in highly proliferative normal tissues including hemopoietic cells [21], the oral mucosa [8] and endometrial tissue from the proliferative phase of the menstrual cycle [22] In the present study, we found that 80% of the tissue samples of head and neck cancers were telomerase positive This is in accordance with previous reports on breast, lung, gastric, head and neck and other malignancies [4–8] A study on squamous cell carcinomas of the larynx reported 89% telomerase positivity [23] Mutirangura et al [15] have reported 87 5% tumour tissues of head and neck squamous cell carcinomas to be telomerase positive While Kagata et al [14] have reported 67% of the tumour tissues to be telomerase positive Twenty percent of tumour tissues from head and neck cancer patients were telomerase negative This supports the earlier findings that there must be some other telomerase-free mechanism for acquiring proliferative capacity [24] It is believed that chromosomes manage to acquire telomeres by recombination with other chromosomes [24]

Moreover, we found that all the precancerous/benign lesions were telomerase positive This is in contrast to previous reports where only a few specimens of precancerous lesions were found to be telomerase positive and identified as high risk group for developing cancer [15] However, in our study, out of 11 patients with precancerous/benign lesions, 4 patients later developed cancer Interestingly, 1 patient had recurrent leukoplakia He first developed leukoplakia in 1992 and was treated with LASER application He developed leukoplakia again in 1996 and then in 1998 On the contrary, another patient with leukoplakia showed slightly positive telomerase activity in the tissue sample There were also 3 patients with haemangioma of tongue These facts could possibly account for 100% telomerase positivity seen in the group of patients with precancerous/benign lesions However, further studies on larger number of patients with precancerous/benign lesions are required to come to specific conclusions.

The current investigation found a surprisingly high number of adjacent normal tissues to be telomerase positive Recently, many authors have reported telomerase positivity in adjacent normal tissue in different types of cancers [8,25,26] However, the incidence of telomerase positivity in adjacent normal tissue is much less than that found in the present study Head and neck carcinogenesis is believed to be a process of field cancerization [27] Repeated exposures of the carcinogenic insults (e g tobacco) to the head and neck mucosal cells, increases the risk for development of multiple independent premalignant and malignant foci [27] Tobacco consumption is believed to be the main etiological factor for head and neck carcinogenesis in India Rate of tobacco consumption is particularly high in India [28,29] Chronic tobacco exposure may bring about genetic and biochemical changes in normal mucosal cells, even though these cells may not yet be histopathologically malignant [27] This might explain the occurrence of high telomerase positivity in adjacent normal tissues as well as the precancerous and benign

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