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Quantitative Estimation of Telomerase Activity in Normal and Premalignant Lesion of Oral Mucosa

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Abstract: ***Introduction:*** *Telomerase, an RNA dependent DNA polymerase, directs the synthesis of telomeric DNA repeats and compensates for the loss of telomeres during cell division, provides the molecular basis for unlimited proliferative potential. This telomerase is up regulated in many carcinomas.*

Objectives: *Present study was aimed at the quantification of telomerase activity in normal oral mucosa and in oral premalignant lesion (leukoplakia) and the comparison of telomerase activity in the two groups. As it is difficult to predict the chances of malignant transformation of oral premalignant lesions only on the basis of dysplasia, an attempt has been made to explore the role of telomerase as a useful prognostic marker.*

Materials and method: *A total of 20 normal oral mucosal samples and 30 mucosal samples of oral premalignant lesion (leukoplakia) were procured from the Dept of Oral Medicine and Radiology, SDM College of Dental Sciences, Dharwad. Quantification of telomerase activity was done using PCR based TRAP protocol and Total Phoretic Lab Software (U.K.).*

Results: *Statistical analysis revealed statistically significant difference in telomerase activity in normal group and premalignant lesion (leukoplakia) group. However, within the comparative group of mild, moderate and severe dysplasia, no statistical significant difference was obtained.*

Conclusion: *The present study revealed that telomerase activity is significantly higher in premalignant lesion (leukoplakia) group than in normal group suggesting that telomerase can be used as an additional prognostic marker. Telomerase levels showed no statistical significant difference in mild, moderate and severe dysplasia subgroup which could be attributed to smaller sample size and unequal distribution of specimens with different grades of dysplasia.*

Further studies with larger sample size and equal distribution of specimens with different grades of dysplasia could be done for substantiation of these results.

Keywords: *Telomerase; Oral premalignant disorders; Quantification; Oral leukoplakia; Prognostic marker.*

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I. Introduction

Wide arrays of premalignant lesions and conditions have been implicated in the development of oral cancer [1]. The most commonly encountered premalignant lesion includes leukoplakia. Studies have shown that between 16 and 62% of oral carcinomas are associated with leukoplakic lesions when diagnosed [**2]** and an Indian house-to-house survey showed that about 80% of oral cancers were preceded by oral pre-cancerous lesions or conditions [**3].** The estimated reported prevalence of oral leukoplakia, worldwide, is approximately 2% [**4].** However, when viewed in relation to an annual malignant transformation rate of 1%, this prevalence figure would result in development of oral cancer in 20 per 100,000 populations per year. Obviously, this cancer incidence figure, based on malignant transformation of oral leukoplakia alone is much too high. These leukoplakic lesions presents either with various degrees of epithelial dysplasia or no dysplasia. The presence of epithelial dysplasia is generally accepted as one of the most important predictors of malignant development in pre malignant lesions [**5].** It should be recognized that some of these dysplastic lesions may remain clinically unchanged or may even show complete regression. Furthermore, carcinomatous transformation may also take place in non-dysplastic leukoplakia [**6].** So these clinical and histologic features alone cannot accurately predict whether premalignant lesions of the oral mucosa remain stable, regress or progress to malignancy. Identification

of molecular markers which can predict disease progression is necessary to improve the management of such lesions [**7].** Even with the availability of various molecular markers there is still no single marker or set of markers that reliably predicts the malignant transformation of leukoplakia in an individual patient.

The discovery of enzyme telomerase and its subunits has led to major advances in understanding the mechanism of cellular proliferation, immortalization, aging and neoplastic transformation. Telomerase is a ribonucleoprotein enzyme that synthesizes telomeres, which are specialized structures at the ends of human chromosomes. Their possible functions include prevention of chromosome degradation, end to end fusion, rearrangements and chromosome loss [**8].** The length of telomere in normal somatic cells progressively shortens with each cell division and this loss of telomere causes cell senescence. Telomerase, an RNA dependent DNA polymerase, directs the synthesis of telomeric DNA repeats and compensates for the loss of telomeres during cell division, universally provides the molecular basis for unlimited proliferative potential.

Telomerase consists of two main components: one is functional RNA component (hTR or hTRC) which serves as templates for telomeric DNA synthesis and other is a catalytic protein (hTERT). hTR is highly expressed in all tissues regardless of telomerase activity with cancer cells having 5 times expression. In contrast, the expression of hTERT is generally repressed in normal cells and unregulated in immortal cells, suggesting that hTERT is the primary determinant for the enzyme activity [**9].**

Since the discovery of telomerase, various studies have been conducted to detect its activity in various carcinomas, body fluids and premalignant lesions. Few studies have reported the absence of connection between telomerase and cancer and that telomerase activity occurs in normal regenerative cells**, [10 & 11]** many other clinical studies show a direct association between telomerase and cancerous cells, body fluids of cancer patients and premalignant lesions [**12, 13**, **14, 15 & 16].** The most commonly used system for detection and quantification of telomerase enzyme activity is the PCR based assay known as TRAP assay. TRAP-ELISA is another technique but because of variation in enzyme kinetics and insufficient amount of sample tissue available, this is not used much [**17].** Another method includes expression of hTERT mRNA but it is only an indicator of expression level of telomerase mRNA and not direct assay for presence of active telomerase.

In the present study an attempt has been made to quantify the telomerase activity in premalignant lesions, oral leukoplakia in particular, using TRAP assay and correlate this telomerase activity with grades of dysplasia. This quantification and correlation between telomerase activity and grades of dysplasia of leukoplakia may have great utility in early diagnosis and prognosis of such premalignant lesions of oral mucosa.

II. Materials and Methodology

For the present study a total of 30 subjects were selected from the clinically diagnosed cases of oral leukoplakia with histological confirmation of dysplasia. From these cases incisional biopsy using 8mm punch is performed. All of these tissue samples were collected prior to any form of treatment given for pre-malignancy. A group of 20 normal oral mucosal tissues obtained from healthy individuals without any habits (smoking, quid chewing and alcohol consumption) who were posted for third molar impaction surgeries. Demographic data and clinical details of each patient was also collected and recorded in a proforma. Simultaneous with the collection of tissue samples for the TRAP assay, bits from each of the tissue samples were fixed in buffered formalin and processed for routine histopathological examination. Patients who were on long term NSAIDs therapy or corticosteroid therapy or on any form of treatment for oral leukoplakia were excluded from the study as they are known to inhibit telomerase activity. A written informed consent was obtained from all the patients. A clinical examination was performed for patients followed by punch biopsy in both groups. The specimens were subjected to histopathological examination followed by estimation of telomerase activity.

TRAP Assay: Immediately after performing the punch biopsy from the lesion, a bit of tissue from the biopsy was fixed in formalin for histopathological examination. Rest of the specimen was directly kept in RNA stabilizing buffer and transported immediately to laboratory in coolant packs to maintain the cold chain. In the laboratory, tissue was homogenized with the ice cold lysis buffer (1 ml) and incubated on ice for 30 minutes. Lysis buffer used for homogenization of tissue was prepared using the following chemicals –10 mM/L – Tris HCl (pH 7.5),1 mM/L – MgCl2, 1 mM/L – EDTA, 0.1mM/L – PMSF, 5 mM/L – â-mercaptoethanol, 5gm/L – CHAPS,100ml/L – glycerol. After incubation, the extracts were centrifuged (Eppendorf Centrifuge 5810R) at 12,000 x g for 30 minutes at 4 0C. The supernatant was aliquoted, rapidly frozed and immediately stored at – 80 0C (In – 80 0C deep freezer).Supernatant of the tissue extracts were then subjected for protein extraction. Protein was estimated in all the extracts using spectrophotometric method. A spectrophotometer transmits a light over a set wavelength range and the absorbance maxima on a digital readout can be observed. In the study Biophotometer plus (Eppendorf) was used. 100microlitre of saline in a civette was run as a blank and values of samples compared to blank were noted at 280 nanometer wavelength. Telomerase activity was estimated at a protein concentration of 20 microgram in the extracts.

Primers used in the TRAP assay:

Cx Primer: 5’ – CCC TTA CCC TTA CCC TTA CCC TAA 3’

Ts Primer: 5' – AAT CCG TCG AGC AGA GTT 3'

TRAP Assay System:Amplified products were manually generated by the TRAP assay using the following protocol.

Telomerase Extension Reaction: Two µl of cell lysate is added to 23 µl of a solution containing 1X PCR buffer, 200 µmol/L dNTP's (50 µmol/L each), and 200 ng of the telomerase substrate (TS primer). The solution is incubated at 30°C for 30 minutes.

PCR Amplification Step: To the extension reaction, 25 µl of a solution containing 1X PCR buffer, 200 µmol/L dNTPs, 3.75U Taq polymerase, 100 ng of the reverse CX primer and 10 ng of the Internal TRAP Assay Standard (ITAS) was added. PCR for the TRAP assay was carried out in a RT PCR Thermal Cycler (Corbett Research) using the following program: 95°C for 15 minutes; 36 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds; 72°C for 5 minutes; 4°C hold.

Procedure: For control reaction:5 µl of control RNA Template (1 µg) was mixed with 4µl of Control Reverse Primer and 11 µl of DEPC water was added to the reaction mix (RNA-Primer Mix). The above mix was incubated at 65 0C for 10 min and immediately chilled on ice. The Control RT PCR reaction was set by adding the components in the following order:Add 25µl Reaction mix provided,Add 20µl of RNA-Primer Mix, 2µl of Forward primer and add 2µl of enzyme mix.Place the above Reaction mix in a thermocycler set to above mentioned conditions.

For test reaction: The Test reverse transcriptase reaction was set by adding the components in the following order.RNA – 2.5µl, Reverse Primer - 1µl, DEPC water – 6.5µl, Total - 10µl. The above mix was incubated at 65 0C for 10 min and immediately chilled on ice.

The Test RT PCR reaction was set by adding the components in the following order:Reaction mix – 12.5µl,Enzymes - 1µl, Primer(TS) - 1µl and DEPC water - 1µl.The above reaction mix was placed in a thermocycler to above mentioned condition.10µl of this PCR product was loaded and run on 12 % polyacrylamide gel at 100 bp at an interval of 10 bp using Gel electrophoresis apparatus. The results were interpreted using UV transilluminator and Gel documentation system (Bioimaging systems).The telomerase activity was determined as positive and negative when compared to the control of known telomerase activity.The quantitative value for telomerase activity was derived using Total Lab Phoretic Software (U.K.) by feeding the ladder pattern of 12 % polyacrylamide gel and compared with the control where value of Telomerase activity was 1µg.(figure 1)

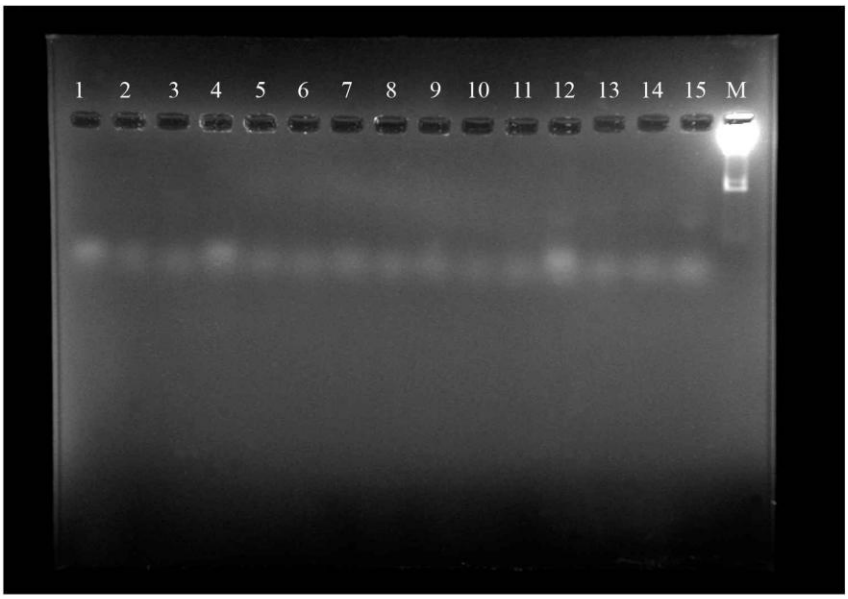


Figure 12: Telomerase activity in tissue extracts
M indicates a 10–base pair (bp) marker;
Lane 1-12 – Extracts of oral leukoplakia
Lane 13-15 - Extracts of normal oral mucosa
Lane 4,5,6-Samples with mild dysplasia
Lane 1,3,7,8,9,11,12- Samples with moderate dysplasia
Lane 2,10,-Samples with severe dysplasia

III. Results and observations

Following the quantification estimation of Telomerase activity for normal group and leukoplakia group, statistical analysis was performed to assess the difference in the Telomerase levels in both the leukoplakia group and normal oral mucosa group.

The leukoplakia group was further divided into 3 subgroups based on histopathology that is mild, moderate and severe dysplasia group (Table 1).

Table No. 1 The number of subjects in each group.

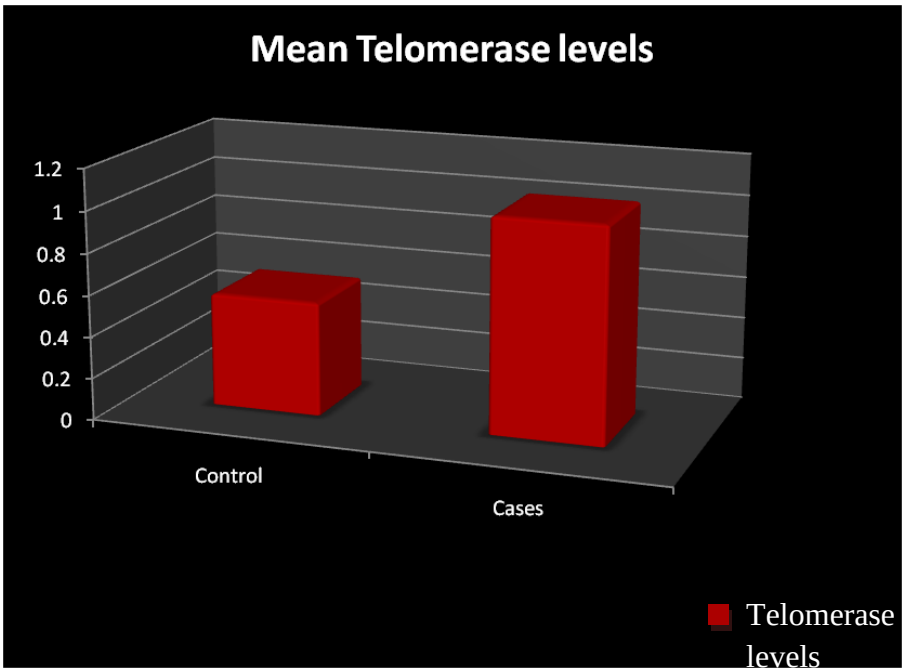
Group	**Number of subjects**
Controls (normal subjects)	20
Cases (leukoplakia subjects)	30
Subgroups in cases	
Mild dysplasia	11
Moderate dysplasia	14
Severe dysplasia	5
Total	30

Descriptive data: Total number of patients in the control group was 20. The minimum telomerase level was .21µg, maximum level was 1.09µg with mean value being .5475µg with standard deviation of .2797.Total number of patients in the cases group was 30. The minimum telomerase level was .32µg, maximum level was 3.16µg with mean value being 1.0307 µg with standard deviation of .1272. In mild dysplasia group (11 cases) the minimum telomerase level was .51µg, maximum level was 2.60µg with mean value being 1.1673 µg with standard deviation of .7401. In moderate dysplasia group (14 cases) the minimum telomerase level was .52µg, maximum level was 2.61µg with mean value being 1.1671 µg with standard deviation of .7402. In severe dysplasia group (5 cases) the minimum telomerase level was .29µg, maximum level was 1.89µg with mean value being .8840 µg with standard deviation of .6909.

T – Test was performed to compare the telomerase levels in two groups i.e. group 1-controls and group 2- cases (leukoplakia). The telomerase levels in control and cases group is statistically significant (P= 0.001) (Table 2) (Graph 1).

Table No. 2 Comparison of telomerase levels in normal and leukoplakia group

	Groups	Number	Mean	Standard deviation	Standard error of mean
Telomerase levels	Control	20	.5475	.2797	6.255E-02
	Cases	30	1.0307	.6966	.1272



Graph No.1: Bar diagram depicting means of telomerase levels in normal(control) and leukoplakia(cases) group

Non parametric test, Kruskal- Wallis test was performed to analyze the difference in telomerase levels within groups that is between controls, mild dysplasia group, moderate dysplasia group and severe dysplasia group. Following ranking by Kruskal Wallis test, chi square test was performed to assess the telomerase levels within groups and to look if any difference occurs in telomerase levels (Table 3).

Table No. 3: Kruskal- Wallis test for Ranks in normal group and subgroups

	Grade	Number	Mean rank	Statistic
Telomerase levels	Mild dysplasia	11	33.41	Chi –square : 9.420 (telomerase level)
	Moderate dysplasia	14	29.89	
	Severe dysplasia	5	24.60	
	Controls	20	18.30	Df: 3
	Total	50		Significance: 0.024

Chi square test showed that telomerase level was 9.420 µg with p value = .024 which is shows that significant difference in telomerase levels between groups exist as p value was less than .05.

To further evaluate the above difference in telomerase levels in various subgroups (based on degree of dysplasia), individual groups were compared separately with each other using Mann Whitney Test which showed that P value was less than .05, therefore the difference in telomerase levels in control group and mild dysplasia, control group and moderate dysplasia and, control group and severe dysplasia is significant, telomerase levels were significantly higher in dysplasia groups than controls.(table 4, 5, 6,)

Control with mild dysplasia (table 4)

Table No. 4: Mann Whitney test for ranks in normal and mild dysplasia subgroup

	Groups	Number	Mean rank	Sum of ranks	Test statistics
Telomerase levels	Control	20	12.63	252.50	Mann-Whitney U-42.500(telomerase level)
	Mild dysplasia	11	22.14	243.50	Z--2.790
					Asymp. Sig. (2-tailed)- .005
					Exact Sig. [2*(1-tailed Sig.)]- .004(a)

Control with moderate dysplasia (table 5)

Table No. 5: Mann Whitney test for ranks in normal and moderate dysplasia subgroup.

	Groups	Number	Mean rank	Sum of ranks	Statistics
Telomerase levels	Control	20	14.23	284.50	Mann-WhitneyU-74.500 (telomerase level)
	Moderate dysplasia	14	22.18	310.50	Z--2.294
					Asymp. Sig. (2-tailed)- .022
					Exact Sig. [2*(1-tailed Sig.)]- .020

Control with severe dysplasia (table 6)

Table No. 6: Mann Whitney Test statistics for normal and severe dysplasia group

	Groups	Number	Mean rank	Sum of ranks	Statistics
Telomerase levels	Control	20	12.45	249	Mann-Whitney U-39.000(telomerase level)
	Severe dysplasia	5	15.20	76	Z--0.748
					Asymp. Sig. (2-tailed)- .454
					Exact Sig. [2*(1-tailed Sig.)]- .488

Telomerase levels were compared between mild, moderate and severe dysplasia group but no statistically significant values were achieved.

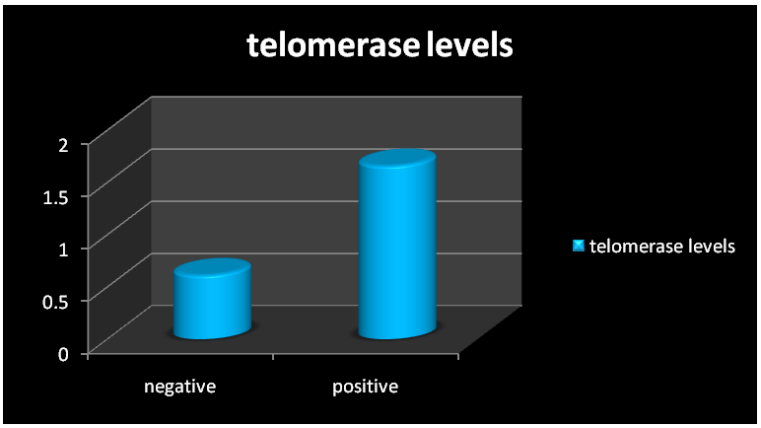
Based on intensity of bands in polyacrylamide gel and comparing it with the control of known quantity of telomerase activity, positive (>1µg) or negative (<1µg) telomerase activity was given. Further the intensity of these bands was compared using total phoretic lab software to get the quantitative values. In the control group all sample showed negative telomerase activity except one. In the cases group 18 showed negative activity on polyacrylamide gel and 12 showed positive activity. So the comparison was done between telomerase levels and positive and negative values in the cases group.(table 7 graph 2)

Table No. 7 Mann Whitney Test comparing levels of telomerase in positive andnegative samples in leukoplakia group

	Groups	Number	Mean rank	Sum of ranks	Statistics
Telomerase level	Negative	18	10.56	190	Mann-Whitney U-19(telomerase level)
	Positive	12	22.92	275	Z--3.770
	Total	30			Asymp. Sig. (2-tailed)- .000
					Exact Sig. [2*(1-tailed Sig.)]- .000

These values were highly significant as p value was less than .05.

Bar diagram showing the mean levels of telomerase in negative and positive subgroups in cases group (Graph 2)



Graph No 2. Mean levels of telomerase in negative and positive subgroups in leukoplakia group.

Further Correlations between different variables like age, chewing and smoking habits and telomerase levels in cases group was also assessed but none of the values reached statistical significance as values for Pearson correlation was more than .01(table 8).

Table No. 8: Correlations between different variables and telomerase levels

		Age	Telomerase levels	Smoking	Chewing
Age	Pearson Correlation	1.000	-.019	.396	-.100
	Sig. (2-tailed)	.	.920	.129	.684
	N	30	30	16	19
Telomerase levels	Pearson Correlation	-.019	1.000	-.105	-.285
	Sig. (2-tailed)	.920	.0	.698	.237
	N	30	30	16	19
Smoking	Pearson Correlation	.396	-.105	1.000	-.408
	Sig. (2-tailed)	.129	.698	.0	.422
	N	16	16	16	6
Chewing	Pearson Correlation	-.100	-.285	-.408	1.000
	Sig. (2-tailed)	.684	.237	.422	.
	N	19	19	6	19

IV. Discussion

Although the diagnosis of precancerous disorders begins with the clinical examination it is the histopathological study which determines the presence and degree of any dysplasia thus providing information regarding the risk of lesion becoming malignant. But the clinical and histological features alone cannot accurately predict whether such a lesion would remain stable, regress or progress to malignancy. Some of such lesions even without dysplasia or mild dysplasia may progress to malignancy whereas other lesions even with severe dysplasia may remain stable and show no transformation to malignancy. Such behaviour of premalignant disorders necessitates the identification of molecular markers which can predict the disease progression. Telomerase is one such molecular marker under research.

Telomerase, a ribonucleoprotein enzyme, is necessary for the immortalization of cells as it catalyzes the replication of telomere repeats. Since it is required for the maintenance or extension of telomere length therefore its expression is increased in transformed cells. Telomerase activity is generally not found in normal tissue, but occurs in low levels in pre-cancerous lesions and in cancer.

Therefore, the current study involves the quantitative estimation of telomerase activity in biopsy specimen of normal individuals and in individuals having leukoplakia and the comparison of telomerase activity between them.

In the present study 20 normal oral mucosa and 30 leukoplakia tissue samples was assessed quantitatively for telomerase activity. In normal mucosa mean telomerase activity was 0.5475µg with standard deviation of +- 0.2797. In leukoplakia group the mean telomerase activity was 1.0307µg with standard deviation of +- 0.6966. The difference in the telomerase activity in control and leukoplakia group was significant as p=0.001(p<.05 was significant).

As far as our knowledge goes only a handful of studies were done on oral leukoplakia. The milestone studies involve; Mutirangura et al study in 1996 [**16]** where they qualitatively estimated telomerase activity in 23 leukoplakia tissues. This study was followed by study of Kannan et al in 1997 [**18]** where they qualitatively

estimated telomerase activity in 36 leukoplakia specimens. Sumida et al in 1998 [**19]** semi quantitatively estimated telomerase activity in 7 dysplastic lesions along with carcinoma lesions. Following this Shimamoto [**20]** and Zhang et al in 2001[**21]** and Fujita et al in 2004 [**22]** quantitatively estimated telomerase activity in 10, 17 and 11 dysplastic lesions along with other benign and malignant lesion. Liao et al in 2000 [**8]** qualitatively estimated telomerase activity in 22 oral leukoplakia lesions. Compared to such studies in the past our study is the first study in which quantitative estimation was done in oral leukoplakia tissue with dysplasia. Based on intensity of bands in 12% polyacrylamide gel and comparing it with the control of known quantity of telomerase activity, positive (>1µg) or negative (<1µg) telomerase activity was given. Further the intensity of these bands was compared using Total Phoretic Lab Software to get the quantitative values. In the control group all sample showed negative telomerase activity except one. One sample of normal oral mucosa showed positive telomerase activity which may be attributed to the mitogenic stimulation of lymphocytes causing telomerase up-regulation [**11].**

In the leukoplakia group, 18 showed negative activity on polyacrylamide gel and 12 showed positive activity that is 40% of leukoplakia lesion showed positive telomerase activity. These results were consistent with the results of various studies where positive telomerase activity ranged from 27.7% up to 63%, with tendency 47% to 54% positive telomerase in most studies. The present study showed comparable results with various studies except for the study by Kannan et al [**18]** where 75% of leukoplakia samples were detected as having telomerase activity but only 28% showed high activity. These variations could be attributed to the qualitative nature of the study. Fujita et al [**22]** in their study reported 63.6% dysplastic lesions as having telomerase activity higher than 20U/µgP. But the small sample size (11 dysplastic lesions) could have accounted for this variation in results. In our study we further categorized leukoplakia into 3 subgroups based on degree of dysplasia. In the mild dysplasia group, out of 11 samples 5 samples showed positive telomerase activity and 6 samples showed negative telomerase activity that is 46% samples of the mild dysplasia showed positive activity. In moderate dysplasia subgroup out of 14 samples 5 showed positive activity and 9 showed negative activities that is 36% of sample in this subgroup showed positive activity. In severe dysplasia subgroup 40% of samples showed positive activity (2 out of 5 samples showed positive activity). The percentage of samples showing positive activity decreases from 46% in mild dysplasia subgroup to 36% in moderate dysplasia subgroup but then again increased from 36% in moderate dysplasia to 40% in severe dysplasia subgroup. These results even within the subgroups are consistent with the overall results of various other studies where positive telomerase activity in various subgroups ranged from 27.3% to 50% in hyperplasia subgroup to 20 to 83.3% in dysplasia subgroup with an average of 40-60% positive telomerase activity [**8, 16, 18, 19, 21 & 23].** These variations could be accredited to the unequal distribution of the samples in various subgroups. The results of our study in mild dysplasia subgroup were quite similar but in moderate and severe dysplasia subgroups, variations may be attributed to the smaller sample size in the various previous studies. As squamous epithelium has very high cell turnover rate, continuous divisions in the basal cells occur to compensate the loss of superficial cells, resulting in the increase of stem cells for the active self-renewal process [**24].** Cellular kinetic studies have also demonstrated a similarity in cell proliferation pattern between the stem cells of squamous epithelium and hematopoietic tissues [**24].** Thus, the telomerase activity in normal oral mucosa may be due to the presence of these stem cells. This could be the reason for positive telomerase activity in one of normal oral mucosa in our study. Cell kinetic studies have shown that proliferation rate is comparatively higher in the hyperplastic and dysplastic epithelia of the oral leukoplakia and carcinoma [**25].** Reactivation of telomerase can occur during any step of progression of carcinoma and may result in uncontrolled proliferation of cells. As mentioned earlier, lesions even without dysplasia or mild dysplasia may progress to malignancy whereas other lesions even with severe dysplasia may remain stable and show no transformation to malignancy necessitates the evaluation of such biomarkers. The data from various studies and even from our study also indicate that telomerase is not always reactivated during the dysplastic stage. Not all malignant and dysplastic lesions exhibited telomerase activity. A long term follow up study showed that dysplastic oral leukoplakia with high telomerase activity developed carcinoma in due course of 11 months [**26].** In one of the studies, Ki-67 labeling index was significantly higher in leukoplakia with higher telomerase activity than in that with low or negative activity [**8].** Accordingly, leukoplakia with telomerase activity is considered to have more proliferative capacity than that without telomerase activity. These telomerase positive leukoplakia specimens with high proliferative capacity, accounts for the transformation of up to 14% leukoplakia into malignancy.

Although telomerase activity is perhaps the single best molecular analyte for malignancy currently available, the assay can have shortcomings like false positive and negatives. The presence of inhibitors of either the telomerase enzyme or the polymerase used in PCR has been reported to be a potential problem in the analysis of a total protein cell extract, resulting in the generation of false negative assays. However, the inclusion of an internal TRAP assay standard and the susceptibility of the enzyme to RNase degradation of its RNA moiety have helped to validate telomerase activity assay data.

Handling and homogenization of solid tissue specimens may lead to partial degradation of labile RNA and coupled with the low abundance of hTERT transcripts, any loss can be a serious concern, especially if attempting quantification. False positive assays can also be misleading. Telomerase is known to be active in stem cell lineages and the high sensitivity of the PCR-based TRAP assay can produce a positive signal if just a few stem cells "contaminate" the sample. This could be one of the causes for discrepancy of results among various studies along with incongruity of the sample sizes. These discrepancies among various studies can be minimized by increasing the sample size and following the proper technique with minimal handling of tissues.

V. Conclusion

The present study aimed at the quantification of telomerase activity in the normal oral mucosa (control group) and oral premalignant disorders (cases group) and to compare the telomerase activity in two groups. Oral leukoplakia lesions were considered in premalignant disorder group as it is most commonly encountered in oral cavity and has high malignant transformation rate. Incisional Biopsy from these lesions was taken and telomerase activity was estimated using TRAP assay and quantified using total lab phoretic software.

The mean telomerase activity in normal oral mucosa was .5475µg and 1.0307 µg in leukoplakia group. T –test showed that the telomerase levels were significantly higher in leukoplakia group with $p = 0.001$. Non parametric test, Kruskal- Wallis test was performed to analyze the difference in telomerase levels within groups that is between normal, mild dysplasia subgroup, moderate dysplasia subgroup and severe dysplasia subgroup. It showed significant difference in telomerase activity within groups with p value of .024. Mann Whitney test showed that significantly higher telomerase activity in mild and moderate dysplasia subgroup compared to normal. However, there was no significant difference between normal and severe dysplasia subgroup. Also there was no significant difference in telomerase activity between different subgroups in leukoplakia group.

It can be concluded from the present study that telomerase activity is significantly higher in leukoplakia group than in normal group. In leukoplakia group 40% samples were telomerase positive. Leukoplakia with telomerase activity is considered to have more proliferative capacity than that without telomerase activity. These telomerase positive leukoplakia specimens with high proliferative capacity, accounts for the transformation of up to 17.5% leukoplakia into malignancy.

Telomerase levels showed no significant difference in mild, moderate and severe dysplasia subgroup which could be attributed to smaller sample size and unequal distribution of specimens with different grades of dysplasia in the leukoplakia group.

Further studies with larger sample size and equal distribution of specimens with dysplasia could be done for substantiation of these results.

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