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Possible Role of the Basal cells in Diagnostics of Oral Cancer

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Abstract

In India, oral cancer (OSCC) account for 30-40% cancers at all sites. In North-eastern India, where this study was conducted, tobacco related oral cancer is very common. High Mortality due to cancer can be decreased significantly if it detected and treated at the initial stage of the disease. The aim of this study was to assess cellular morphology in a group of patients with oral leukoplakia, erythroplakia and oral cancer measured by means of basal cell frequency in exfoliated buccal cells. The study is the first report on the changes in basal cells associated with oral cancer. The data strongly support a positive correlation between the basal cell level and malignization (changes from pre-malignant stage to cancer). It is suggested that the evaluation of frequency of basal cells in exfoliated buccal cells may be an additional criterion for establishing oral cancer risk and the study of basal cells in buccal smears will increase the sensitivity and specificity of cytology which could impact in diagnostics and secondary prevention of oral cancer.

Keywords: Basal cell, exfoliated buccal cells, premalignant cell, oral cancer.

Introduction

Oral cancer is an important health issue. In North-east India, incidence of tobacco related oral cancers is very high. The morbidity and mortality associated with this disease is a cause of major concern in this region.

Many approaches and techniques have been developed for monitoring human populations that have been exposed to environmental mutagens[1]. Genetic monitoring using cytogenetic endpoints in suspected high risk populations have been carried in several studies[2,3,4] . The study of basal cells opens up a whole new vista into the kinetics and morphological changes in the oral epithelium that can be crucial in biomonitoring studies.

In preneoplastic stages, major mucosal alteration takes place in the epithelium in varying degree according to the severity of dysplasia[5]. Hence, the oral epithelial cells are promising target cells to study the changes that take place in the epithelium. Basal cells are the cells that differentiate and maintain the profile, structure and integrity of the buccal mucosa. The stratum germinativum of the buccal epithelium contains actively dividing basal cells[6]. Basal cell can be easily distinguished from a mature differentiated cell from the nuclear to cytoplasm ratio which is larger in basal cell than that in differentiated buccal cells. Basal cells have a uniformly stained nucleus and the cells are smaller in size when compared to differentiated buccal cells. By studying basal cells valuable information can be obtained on cellular proliferation and cell repair which may reflect related changes in the structural profile of the buccal mucosa.

A considerable number of reports have been published on the incidence of micronucleus in the buccal epithelial cells in premalignant and malignant conditions. However, very few studies have considered the changes in basal cells. Degenerative nuclear anomalies other than micronucleus have been reported to play an important role in toxicity assessment studies[7].

Material and Methods

Characteristics of the participants: The study was approved by Institutional Ethics Committee of Assam University, Silchar. The age range of the participants varied between 27 and 45 years. The exposed group consisted of 30 patients with oral squamous cell carcinoma, 30 patients with oral precancerous lesions (15 with leukoplakia and 15 with erythroplakia) and 30 age and sex matched control group. Participants were asked a structured questionnaire to obtain personal information with regard to chewing and smoking habits, exposure period and health status. After obtaining consent, samples were taken from the buccal mucosa of subjects after diagnosis by a practicing oncologist from the Department of ENT. Only subjects who were not under any radiotherapy or chemotherapy sessions were recruited for the study.

Sampling procedure: Cells sampling was done following the method of Beliën et al. with minor modifications[8]. Pre-moistened cotton swab (Johnson & Johnson, India) were rotated in a circular motion against the inside of the cheek following rinsing of the buccal cavity by water. The cells were washed twice in normal saline and buffer solution through centrifugation. Smears were prepared in pre-cleaned slides, air-dried and fixed prior to staining.

Staining and Scoring: Fixed slides were treated for 1 min each in 50% and 20% ethanol and washed in deionized water for 1 min prior to staining. These were then treated in 5M

hydrochloric acid for 30 min followed by washing for 3 min in running tap water. Moist slides were treated with Schiff's reagent (S-D fine chemicals, Mumbai) at room temperature in dark for 0 min, washed in running tapwater for 5 min and rinsed for1min in deionized water. Slides were double stained in 0.2% light green (Hi-media) for 1 min and rinsed in deionized water for 2 min, allowed to air-dry, mounted in DPX. Coded slides were scored by a single scorer to eliminate inter-observer variation using a light-microscope (Leica DMLS) at 1000 magnification. One thousand fifteen hundred to 2000 mucosal cells were counted per individual to determine the basal cell frequency.

Statistical analysis: One-way analysis of variance (ANOVA) and Student's t-test was used to determine the significance of the cellular parameters using Graph pad Prism software. Significance was accepted at $P < 0.05$.

Results and Discussion

The percentage of basal cells in exfoliated buccal epithelial cells from subjects with oral precancerous lesions and oral cancer is illustrated in figure-1, figure-2 and figure-3. A comparison of frequency of basal cells in all the groups and control group is illustrated in figure-4. Bartlett's test for equal variance was positive and significant at $p<0.0001$. The basal cell percentage for subjects with leukoplakia was 2.81 [$p<0.001$, 95% CI], for subjects with erythroplakia was 2.10 [$p<0.001$, 95% CI,], all of which were significantly higher from control**.** The basal cell percentage for subjects with oral squamous cell carcinoma was 3.91 [$p<0.001$, 95% CI,], Tukey's pair wise analysis revealed significant differences between the patient groups with leukoplakia, erythroplakia and oral squamous cell carcinoma.

Basal cells differentiate to form mature buccal mucosal cells and an alteration in the cell turn over rate indicates an altered condition in cell proliferation kinetics and cell cycle, which is hall mark of carcinogenesis[9,10].

It was observed that basal cell frequency significantly ($P< 0.001$) increased in subjects with leukoplakia, erythroplakia and oral squamous cell carcinoma as compared to the control group. A progressive increase in basal cell percentage in control, premalignant cases and malignant cases was seen. A similar observation was made by Rich et al as the disorder progresses from normal, to dysplasia, to neoplasia[11]. The lining of the epithelial layers become highly distorted with dysplasia and epithelial integrity is lost resulting in the basal cells moving upward to the stratum granulosum and spinosum[12]. The increase in the number of basal cells could also be due to malfunction of cell cycle checkpoint[13]. Thus, the increase in the number of basal cells in buccal epithelial cell is an important parameter indicative of loss of epithelial integrity as well as / or dysregulation of proliferative potential of basal cells as it is distinct from mechanisms characterised by redox regulation[14].

Conclusion

Since a very limited number of reports have so far been published on basal cell frequency in exfoliated buccal epithelial cells in patients with precancerous lesions and conditions, the present findings are important for further research in the field of identification of biomarkers of precancerous lesions and conditions and possibly cancerous lesions also. These promising results need to be replicated in larger studies and in cohorts of other cancer to determine specificity of changes patients with oral premalignant lesions an oral cancer.

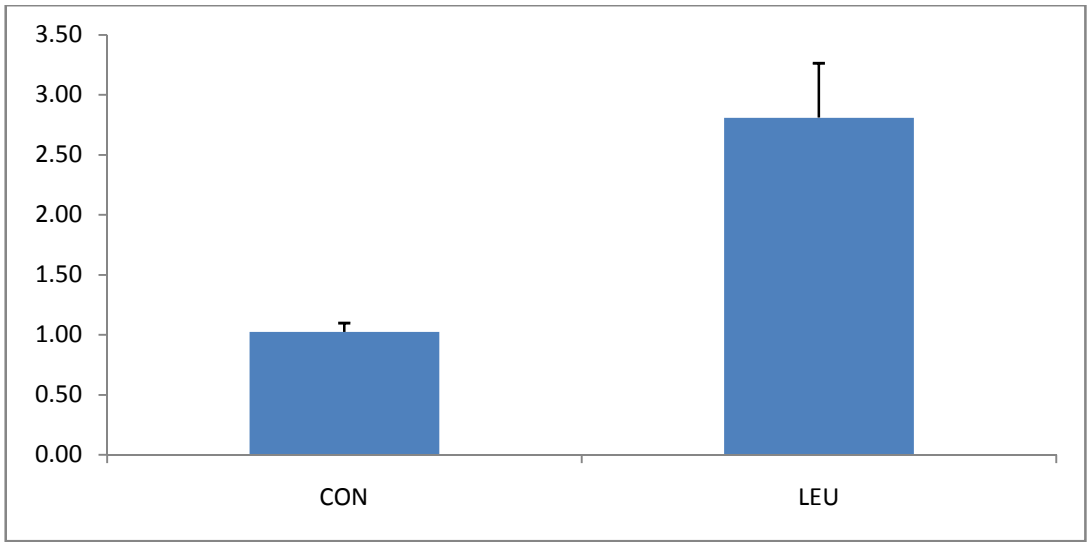

Figure-1
Frequency of basal cells in controls and leukoplakia patients. Values are significantly different from control, ***: P<0.001 Statistical analysis: Student's t-test

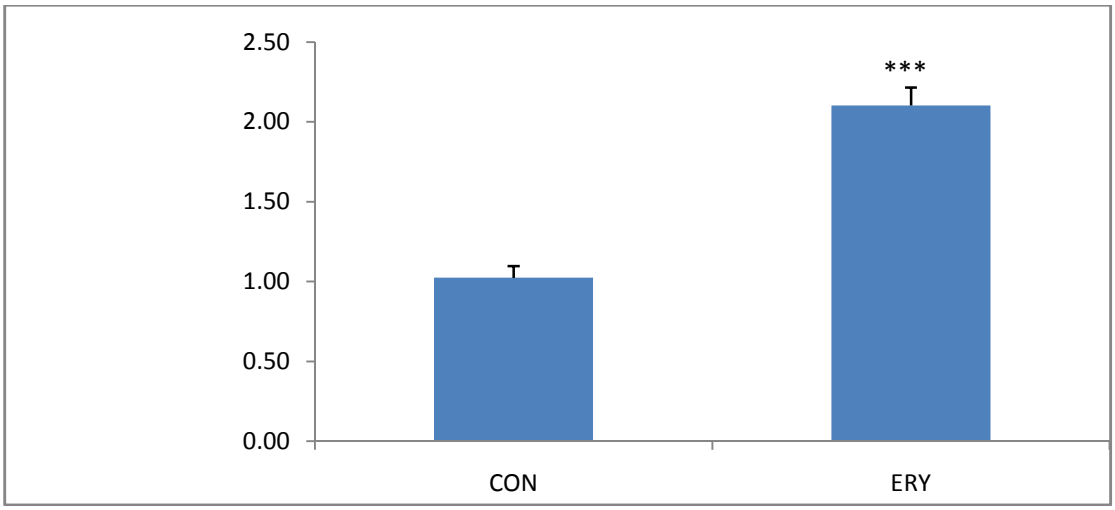

Figure-2

Frequency of basal cells in controls and erythroplakia patients. Values are significantly different from control, ***: P<0.001 Statistical analysis: Student's t-test

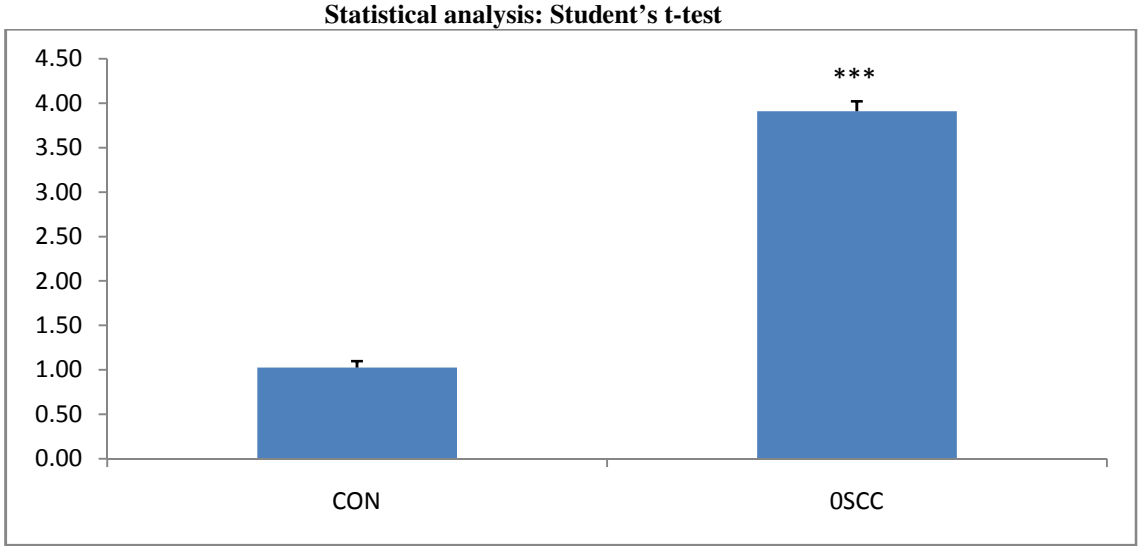

Figure-3

Frequency of basal cells in controls and oral squamous cell carcinoma patients Values are significantly different from control, ***: P<0.001. Statistical analysis: Student's t-test

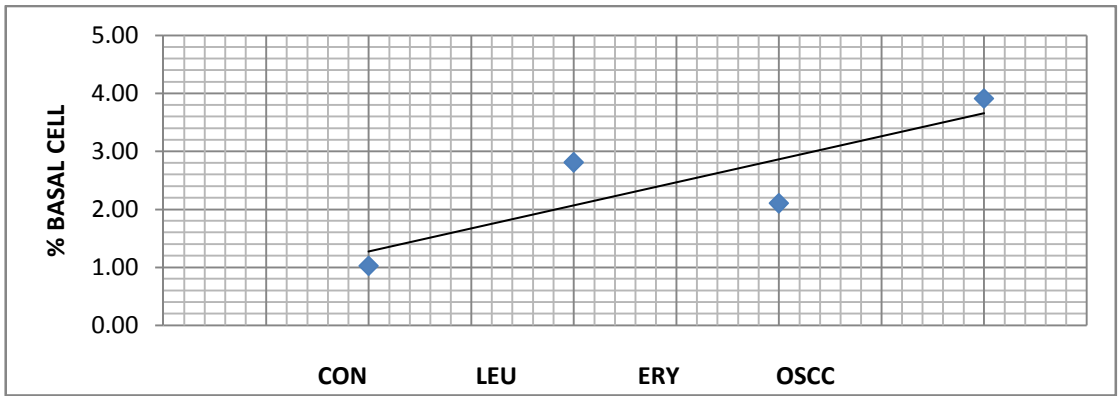

Figure-4

Shows a scatter plot for basal cell frequency biomarker as expressed in control, leukoplakia, erythroplakia and oral squamous cell carcinoma patients

Conflict of interest: The authors do declare that there is no conflict of interest.

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