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ENHANCING DIAGNOSTIC PRECISION: ANALYSIS OF AUTOFLUORESCENCE AND DIFFUSE REFLECTANCE VALUES IN APPARENTLY HEALTHY ORAL MUCOSA OF THE KERALA POPULATION

Dentistry

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ABSTRACT

Background: Oral cancer remains a leading cause of morbidity and mortality in India, largely attributed to habitual practices like tobacco use, betel quid chewing, and alcohol consumption. These habits induce subclinical mucosal changes that are difficult to detect with conventional methods, but newer diagnostic techniques can identify these alterations, offering opportunities to detect carcinoma even before clinical signs appear. Light-based diagnostics such as autofluorescence (AF) and diffuse reflectance (DR) spectroscopy offer non-invasive tools for early detection. **Objective:** To evaluate the correlation of autofluorescence and diffuse reflectance values of the oral mucosa with age, gender, and oral habits across different oral sites, considering the local environmental conditions of Kerala. **Methods:** A cross-sectional study was conducted among 200 apparently healthy volunteers (100 with oral habits and 100 without) at the Government Dental College, Trivandrum. Autofluorescence and diffuse reflectance data were obtained from six intraoral sites using a bimodal multispectral imaging system (BMIS). Statistical analyses included One-Way ANOVA, ANCOVA, independent t-test, and post hoc Tukey's test to assess significance across age, gender, and habit history. **Results:** Statistically significant differences were observed in autofluorescence values at the dorsum of the tongue between habit and non-habit groups ($p = 0.0432$). ANCOVA confirmed this significance even after adjusting for age, highlighting that habits independently influence AF at the dorsum. One-Way ANOVA revealed significant differences in AF at the lateral surface of the tongue ($p = 0.007$) and DR values at the dorsum ($p = 0.031$) and ventral surface of the tongue ($p = 0.010$) across age groups. No significant gender-based differences were observed. Post hoc analysis showed the >60 years group had lower AF lateral tongue values, while the 15–30 group exhibited higher DR ventral tongue values. **Conclusion:** This study establishes important baseline AF and DR values for the Kerala population, showing notable differences associated with age and habitual practices. Non-invasive spectral imaging provides a promising approach to early detection, particularly among high-risk, habituated individuals.

KEYWORDS

Autofluorescence, Diffuse Reflectance, Habitual Risk Factors, Oral Cancer Screening

INTRODUCTION

Oral cancer poses a major public health challenge, especially in India, where it ranks among the top three cancers due to prevalent risk factors such as smoking, chewing tobacco, and alcohol use. According to GLOBOCAN 2020, over 377,000 new cases and 177,000 deaths were reported globally, with India contributing a substantial burden[1]. Chronic exposure to carcinogens in these substances leads to molecular alterations in oral mucosa, progressing to potentially malignant lesions and ultimately oral squamous cell carcinoma (OSCC)[2]

While histopathological evaluation remains the gold standard, there is increasing interest in non-invasive diagnostic methods capable of detecting early or subclinical changes. Autofluorescence (AF) and diffuse reflectance (DR) spectroscopy have emerged as valuable optical diagnostic techniques. These technologies detect biochemical and structural tissue changes by analysing light absorption and emission properties, making them effective tools for early diagnosis[3,4].

This study evaluates the correlation between autofluorescence and diffuse reflectance values of the oral mucosa and variables such as age, gender, and oral habits across various oral sites, taking into account the local environmental conditions of Kerala using a bimodal multispectral imaging system (BMIS).

MATERIALS AND METHODS

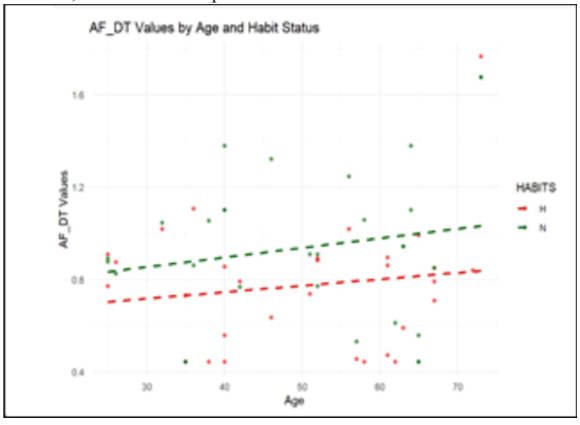
A cross-sectional observational study was conducted, and two hundred apparently healthy volunteers participated, including 100 individuals with habits (tobacco, areca nut, or alcohol use) and 100 without. Inclusion criteria comprised individuals without visible oral lesions or inflammation, and exclusion criteria included any current or past history of oral premalignant or malignant conditions.

Participants underwent imaging with a Bimodal Multispectral Imaging System (BMIS), which uses narrowband LEDs emitting violet, green, yellow, and red light, combined with a sub-miniature monochrome CMOS camera. The device captures both AF and DR data across six intraoral sites: hard palate, dorsum of the tongue, lateral tongue, ventral tongue, left buccal mucosa, and right buccal mucosa.

Data were analysed using SPSS statistical software. One-way ANOVA tested differences in AF and DR values across age groups. ANCOVA was used to assess the effect of habits while controlling for age. Independent t-tests compared male and female participants, and post hoc Tukey's test determined pairwise group differences when ANOVA indicated significance. A p-value <0.05 was considered statistically significant.

RESULTS

Significant differences in AF values were observed between individuals with and without oral habits. At the dorsum of the tongue, AF values were significantly lower in the habit group compared to the non-habit group ($p = 0.0432$). After adjusting for age using ANCOVA, this significance persisted, indicating that the effect was independently attributable to the presence of oral habits and not merely due to age variation, as shown in Graph 1.



Graph – 1- AF values at the dorsum of the tongue significantly differed between habit groups, independent of age (ANCOVA)

Regarding age, One-Way ANOVA revealed a statistically significant difference in the AF values at the lateral surface of the tongue ($p = 0.007$). Similarly, DR values at the dorsum of the tongue ($p = 0.031$) and the ventral surface of the tongue ($p = 0.010$) varied significantly across age groups.

Post hoc analysis with Tukey's test showed that mean AF values at the lateral tongue were lowest in the >60 years group, compared to the 15–30 and 51–60 years groups. Additionally, the 15–30 years group had higher DR values at the ventral tongue than the 31–40 years group. No significant post hoc differences were found for DR values at the dorsum of the tongue.

An independent t-test comparing male and female participants showed no statistically significant differences in AF or DR values across any of the six intraoral sites.

DISCUSSION

This study highlights the diagnostic value of autofluorescence and diffuse reflectance imaging in identifying subclinical alterations in oral mucosa, especially among individuals with high-risk oral habits. The significantly lower AF values at the dorsum of the tongue in the habit group, even after adjusting for age, support existing evidence that tobacco and areca nut constituents cause biochemical and structural tissue changes long before clinical lesions become evident [5,6].

AF detects loss of collagen cross-linking and changes in metabolic cofactors like flavins and NADH, while DR reflects tissue oxygenation and scattering, both of which are altered by chronic mucosal exposure to carcinogens[7]. These parameters provide a real-time, objective assessment of tissue health.

Our findings are especially pertinent for primary screening in high-risk populations. The dorsum of the tongue, showing significant differences in AF due to habits, could serve as a sentinel site for non-invasive surveillance.

Age-related changes were also observed, with lower AF in individuals above 60 years. This may be attributed to natural aging processes that reduce collagen content and tissue autofluorescence, which has been similarly documented in previous studies[8,9]. However, the absence of gender differences in our study suggests that AF and DR are more closely linked to external exposures and ageing than inherent sex-based anatomical variations.

Overall, the findings affirm the potential of BMIS technology for integration into routine clinical workflows, particularly in community settings where biopsy may not be feasible or ethically justified.

CONCLUSION

Ur study revealed significant variations in autofluorescence and diffuse reflectance values associated with age and oral habits. The findings strongly indicate that habitual use of substances such as tobacco and areca nut may lead to subclinical changes in the oral mucosa, even in the absence of clinically visible lesions.

BMIS-based spectral imaging emerges as a valuable tool for early detection, habit-related surveillance, and guiding biopsy decisions. Future studies should focus on patients with premalignant and malignant lesions to validate the predictive power of these spectral markers.

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