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ORIGINAL ARTICLE

Oral Manifestations in Active Consumers of Areca Nut products through Brush Cytology

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ABSTRACT

Aim: To validate oral cellular changes present withinmucous membrane of active consumers of areca nut products through brush cytology

Methods: This proposed study was prospective cross-sectional researchwhich inducted 50 participants with habit of consumption of areca nut products. Oral mucosa brush cytology was recorded in all subjects fulfilling the inclusion criteria and the sample was processedthroughstaining with Hematoxylin& Eosinto observe cellular features. The findings were statistically analyzed through SPSS 23.0

Results: Amongst the sample size of 100, 48(48%) of cases had squamoproliferative activity in oral mucosal membrane of active areca nut products consumers. High statistical significance was appreciated regarding structural changes. In addition, chronic inflammation was also detected in 26(26%) participants of research with a p value of 0.023.

Conclusion: Through brush cytology, more individuals with consumption habit of areca nut products could be screened for suspicious lesions and encountered at a curable stage providing a wider net for early detection. Findings of squamoproliferative activity and inflammatory cells in mucous membrane rings an alarm to start management protocol as early as feasible to counter the disease process well in time and reduce morbidity rate.

Keywords: Inflammatory cells, squamproliferative lesions, areca nut, oral mucous membrane

INTRODUCTION

Oropharyngeal and oral cancers are labelled as a health challenge globally with a calculated incidence of 3 lacs newly reported cases annually while in subcontinent region, prevalence rate ranges from 20 per 100,000 population[1]. Almost 1% of the globe's population has encountered premalignant lesions of oral cavity along with prime involvement of younger individuals which emphasizes the need to identify factors important for prediction of malignant transformation[2]. Delay in diagnosis explains for raised morbidity and mortality and due to this factor almost half of oral cancer conditions are diagnosed as stage III or IV during time of first diagnosis which eventually leads to poor life quality[3].

If oral carcinogenic condition is detected in reasonable time, it can account for improving morbidity of the condition through treatment and better survival rate of up to 82% and if a localized region of oral cavity is identified; Nevertheless, this rate can go down to 32% if metastasis has not initiated. Most of the time, cancerous lesions are preceded by wellacknowledged "oral potentially malignant disorders (OPMDs)"[4].These group of disorders consist of conditions affecting oral mucous membrane with high chances of disrupting the normal anatomy of oral mucosa. There could be diverse clinical presentation of oral potentially malignant disorders. (OPMD). While evolving, they may represent visible changes with respect to oral mucosa thickness and color alteration[5].

Various significant findings through scientific literature in various epidemiological surveys, cohort studies, case control, in vitro and interventional studies suggest usage of areca nut in developing OPMD simultaneously. These two share a clear dose dependent relationship for its frequency and time duration of chewing activity along with area of placement[6]. There is adequate evidence in literature verifying that only by visual inspection, early oral cancers and precancers cannot be differentiated from benign oral cavity lesions, regardless of clinician's expertise. Therefore, oral symptoms without an underlying etiology should not only be observed but evaluated further to rule out dysplasia or carcinogenic changes[7]. Bacterial infection is one of the prime causes for chronic inflammation which facilitates cellular changes such as squamoproliferative activity, angiogenesis, mutagenesis, and oncogenic changes that lead to develop oral carcinoma[8]. It is important to differentiate carcinoma from multiple other benign and reactive squamoproliferative changes to avoid setbacks through diagnostic pitfalls[9]. For management of oral potentially malignant disorders, it is worthwhile to detect them timely and identify early occurring abnormalities in squamous cells of oral mucosa[10]. One such detection protocol is exfoliative cytology, which serves as a quick, minimally invasive procedure, painless, well tolerated by all patients and efficient to collect cells adequately[10,11].

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MATERIALS AND METHODS

This study was conducted on 100 clinically suspected and diagnosed cases for oral potentially malignant disorder. Institutional committee for ethical review approved the research. Subjects inducted were considered regardless of age and gender. Inclusion criteria were the subjects with active habit of consumption of Areca Nut products.A commercially available soft bristle toothbrush was used to retrieve sample from oral mucosal surface using moderate to severe pressure by hand. Brush was repetitively brushed in same direction to obtain squamous epithelial cells. Sample from brush was then immediately transferred to the middle third area of a labelled glass slide and fixed with 100% ethanol for staining with Hematoxylin and Eosin. Preserved smears were sent to a qualified pathologist for evaluation. As this type of biopsy produces scanty cellular data, only slides with more than 20 viable cells were selected to observe cellular changes to retrieve acceptable and optimal results.

RESULTS

In this research, a total of 100 samples were evaluated for abnormal cytology suspecting squamoproliferative lesion and chronic inflammation.Squamoproliferative lesion was detected in 48 individuals through pathological investigation. These findings emphasized on further investigation of detailed histological

analysis to rule out carcinogenic changes. There were 52(52%) of cases as negative for squamoproliferative lesion. p value was calculated to be highly significant (0.002). Table 1 shows frequency distribution of squamoproliferative lesion (Fig. 2). Regarding chronic inflammation, a total of 26(26%) of samples were positive for chronic inflammatory cells (p value 0.023). The sample statistics of chronic inflammation are summarized in table 2 and figure 1 illustrates aggregation of inflammatory cells. All the results were validated using Chi square analysis as all categorical variables were analyzed.

Table 1

Squamoproliferative lesion	Detected	Undetected	P value
Frequency Percentag	48(48%)	52 (52%)	0.002

Table 2

Clinical inflammation	Detected	Undetected	P value
Frequency Percentage	26(26%)	74 (74%)	0.023

Fig.1: Cytological micrograph illustrating pleomorphism in squamous cells and aggregation of multiple inflammatory cells (Black circles)

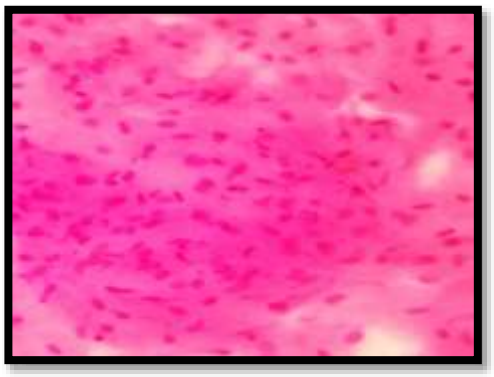
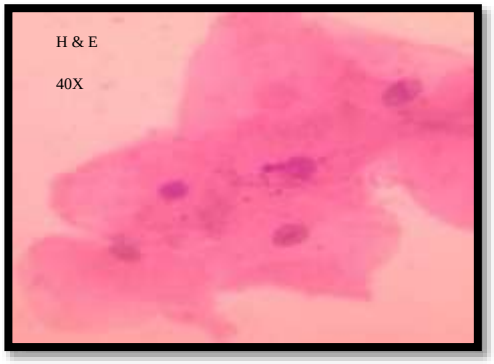


Fig. 2: Cytological micrograph showing squamoproliferative cells of oral mucous membrane indicating progression into malignancy



DISCUSSION

Early inspection for premalignant or malignant lesion promises to upgrade the survival and morbidity rate of the disease. Cytological examination serves as a non-aggressive procedure having broad potential to overcome the diagnostic gap that presently challenges the prognosis and early detection of disease[12]. Persistent use of areca nut and other betel quid associated products and exposure of oral mucosa to carcinogenic compounds promote bacterial mutagenicity. This induces chromosomal aberrations, derangement of epithelial cells adapting cellular changes of pleomorphism, hyperchromatism promoting abnormal mitosis amongst oral mucosal cells[13].

Exfoliation of cells through brush cytology holds a valuable role in diagnosing certain local and systemic diseases. Various events occurring in the human body are reflected in oral cavity through variable findings in cytomorphology of exfoliated cells.In cases of malignancy deeper tissue cells also become loose and shed along the superficial cells. This phenomenon brings ease to spot abnormal cells in brush cytology[14]. Our research found variations in normal anatomy of squamous cells of the oral cavityhighlighting squamoproliferative activity indicating of abnormal mitosis. Similar research by Alsarraf, Kujan & Farah (2018) also evaluated clusters of squamous cells with marked atypia, necrotic material, bacterial plaque, and inflammatory cells alongside oral mucosal squamous cells[15].

In our research, we have categorized squamoproliferative lesion and inflammatory cells based on cytopathology. Similarly, criteria by Papanicolaou (1960) also categorizes premalignant and malignant lesionsbased on cytological changes. Scheifele et al., also has emphasized usage of brush cytology for appreciating variations at molecular level and dysplastic cells for recording histology even for the benign lesions of the oral cavity.[16]Research has proven that just as visual inspection of utrine cervix is insufficient to identify precancers or cancers. Similarly, inspection only clinically has also proven to be extremely doubtful in validating presence of precursor lesions and early carcinoma. Although Papanicolaou is a screening test for mass population without knowing underlying disease and brush cytology could be utilized as a diagnostic test for viable abnormalities but also serves to aid the clinical examination.[17]Our research showed statistically significant results of association of inflammatory cells and squamoproliferative lesions in areca nut product chewers. Research by Aparaajita & Mundra (2018)also found suspicious malignant cells in consumers of ill habits such as tobacco, smoking and alcohol[18]. A diseased cell also shows degenerative changes of nucleus such as karyolysis which occurs subsequent to pyknosis, shrinkage, condensation of chromatin along with pyknotic nucleus fragments. All these microcellular features lead towards carcinoma eventually[19].

CONCLUSION

Early detection of oral premalignancy or malignancy becomes advantageous by usage of painless, quick, noninvasive, and patient compatible procedure of brush cytology.Clinicians should enforce brush cytology as a chairside diagnostic technique in all areca nut product consumers. Changes in structure of squamous cells can facilitate to assist in predicting the exact status of basic defects present at cellular level. Further studies with larger sample size and quantitative details of cells would be beneficial to understand the pathognomic changes in areca nut products consumers.

Conflict of interest: All authors declared no conflict of interest.

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