Original Article

COMPARISON OF EXFOLIATIVE CYTOLOGY AND INCISIONAL BIOPSY HISTOPATHOLOGY DIAGNOSING ORAL LEUKOPLAKIA'S IN SINDH HYDERABAD, PAKISTAN

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ABSTRACT:

BACKGROUND & OBJECTIVE: The malignant neoplasms especially oral leukoplakia's are recognized as a complex tumor with unique morphology. Current study aims to evaluate 182 clinically confirmed cases of oral leukoplakia with complete demographics. Where the major study objectives include; assessment of comparative sensitivity and specificity of exfoliative cytology diagnosing pre-malignant lesions.

METHODOLOGY: Current cross-sectional, comparative histopathological study was carried out at Department of Dentistry-Isra Dental College, Isra University, and Liaquat University Hospital Hyderabad. About 182 cases of oral leukoplakia and 87 control healthy participants were enrolled in the study for exfoliative and incisional biopsy examination.

RESULTS: The subjects were categorized into Group-I (182) patients and in group-II (87) healthy subjects. Males were at greater risk of oral leukoplakia (72.5%) as compared to females (27.5 %). Age group 31-40 year with smoking history was more prone to oral leukoplakia's (30.2%). Where buccal mucosa and tongue-based site-specific leukoplakia lesions were common in Group-I patients. From Group-I about 37.5 % cases of oral leukoplakia were identified as mild dysplasia and only six (3.3%) cases were as severe dysplasia. About 8.5 % cases were negative for AgNOR staining. The sensitivity analysis revealed 71.4% sensitivity of exfoliative cytology.

CONCLUSION: Current study declare 71% sensitivity and 93% specificity of exfoliative cytology for the diagnosis of oral leukoplakia's in routine settings. Where in advanced settings the outcomes may be more promising.

KEY WORDS: Oral Leukoplakia, Exfoliative cytology, Cancer diagnosis.

INTRODUCTION:

Recent research reports highlighted a significant rise in malignant neoplasms including Oral squamous cell carcinomas (OSCC) [1–3]. The post five-year diagnostic analysis of malignant neoplasms revealed up to 50% survival chances of the patients only [4,5]. In general, the detection of malignant neoplasm is assessed by changes in physical appearance of certain precancerous tissues in comparison to normal bodily tissues [6–8]. The characterization of subjected malignant pre-neoplastic conditions represents leukoplakia, erythroleukoplakia and related tumors [6,9].

Beside above classification the world health organization categorized pre-malignant lesions including leukoplakia into a general category of malignant neoplasms [5–10]. The pre-malignant neoplasms were representing white plaques as different in morphology from rest of cancer types [11]. More specifically, expert oncologist predicted 1.5-2.6 % cases of oral carcinomas stand in the category of oral leukoplakias. Where the chances of malignancy were recorded up to 18% [1,6,11,12,13].

The detailed literature research revealed that, the prevalence of oral leukoplakia was not associated with geographic locations, where significant gender association was reported. A retrospective analysis focusing 23 studies claimed that, males were more prone to oral leukoplakia in comparison to females. Where the annual incidence of leukoplakia associated oral carcinomas ranges 6-29 cases per 100,000 oral cancers reported worldwide [14]. Research reports further elaborated certain risk factors enhancing malignancy of pre-malignant oral lesions and it believed that 16-60 % leukoplakia cases end up with oral carcinomas [6]. Enlightening potential risk factors associated with leukoplakia include, smoking, sex, affected site and patient age as contributing factors. Some studies acknowledged the role of dysplasia as well in leukoplakia transformation [1, 13, 15, 16]. Similarly, Silver staining technique was used to stain leukoplasia tissues. The tissue silver staining basically stains the nucleolar regions of pre-malignant cells. The subjected regions are responsible for ribosome protein production in the cell. The over production of ribosomes protein represents tumor presence

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within the cell [17–18]. Whereas the cellular homeostasis is essential to regulate cell cycle and protein synthetic machinery. The abnormal cellular activities are primary indictors of cellular malignancies, especially greater production of nucleolar regions of premalignant cells. Histological evaluations revealed that, the number of nucleolar regions in pre-malignant cells determine the severity of leukoplakia lesions [19, 20]. Due to precise patterns of leukoplakia transformation, designing specific diagnostic and therapeutic protocols is a challenge [1,11]. Assessment and ranking of existing diagnostic and therapeutic techniques would be a promising approach mitigating leukoplakia transformations into oral carcinomas at greater extent [6]. Current study aims to find the relationship among existing diagnostic approaches adopted for leukoplakia diagnosis in Pakistan. Further, we will also rank the specificity and sensitivity ratios of chosen approaches diagnosing leukoplastic conditions.

METHODOLOGY:

Current cross-sectional, comparative histopathological study was carried out at Department of Dentistry - Isra Dental College, Isra University, and Liaquat University Hospital Hyderabad. The study has been approved by the ethical review committee of Isra University. The approved study duration was approximately one year (From Sept 2017- August 2018). By following multipurpose convenient sampling technique 269 subjects were enrolled in the study. Further, the study participants were categorized into Group-I (oral leukoplakia) and Group-II (control group participants). The participants inclusion criteria include, Patients visiting Department of Dentistry - Isra Dental College, Isra University, and Liaquat University Hospital Hyderabad presenting the symptoms of pre-malignant oral conditions. Whereas Patients with ages above

twenty-year up to seventy years from both genders were only considered. The patient exclusion criteria were declared as; cases other than leukoplakia, confirmed cases of oral squamous cell carcinomas and Patient ages below 20 year and above 70 year were excluded.

The departmental ethical approval was pre-designed and approved by institutional research ethical committee, ISRA Dental College Hyderabad Sindh prior to patient selection and sample size determination. Similarly, patient concerns and willingness to participate in the study was ensured.

In Group-I the suspected cases of oral leukoplakia were tested for exfoliative cytology as well as incisional biopsies were taken for histopathological evaluation by using H & E staining. Where in Group-II normal healthy participants were enrolled for exfoliative cytology only. The cytobrush in parallel with Papanicolaou staining method was employed for exfoliative cytology for both control and experimental groups [21-23]. The histological analysis of leukoplakia cases was confirmed by using Hematoxylin and eosin staining. The significant morphological changes observed in pre-malignant lesions were recorded as epithelial dysplasia and hyperorthokeratosis [24]. Further the data presentation and statistical analysis were made by using Revman5.30, SPPS 22.0 and Microsoft office version 2010.

RESULTS:

Current cross-sectional comparative study based on 269 subjects was further subdivided into two groups. About 182 (67.6%) suspected cases of oral leukoplakia were placed in Group-I. In contrast Group-II constitutes 87 normal healthy participants. In Group-I males were at greater risk of oral leukoplakia (72.5%) in comparison to females (27.5 %). Where the age wise outcomes reveal that, the age group 31-40 years were more prone to oral leukoplakia (30.2%). Following the habitual analysis of oral leukoplakia cases, smoking was recognized as leading factor (30.7%) causing pre-malignant lesions, where buccal mucosa and tongue-based site-specific leukoplakia lesions were common in Group-I patients. The comparative demographic assessment of control group participants in parallel to experimental group participants was also determined. About 44 % control group participants were enrolled ages 31-40 years, with highest percentage of non-smokers 46% (Table-I). All the control participants were operated for buccal mucosal exfoliative cytology for the isolation of epithelial cells in parallel to the oral leukoplakia 182 cases for exfoliative cytology from different sites (Table-I).

Table- I: Demographics of Group-I (Leukoplakia cases) & Group-II (healthy control participants) at 95 % CI (Chi-square test).

Variable	Groups		% Risk Ratio	(n = 269)
	G-I	G-II		
Age Distribution (years):				
20-30	30	22	25.10%	52
31-40	55	38	43.40%	93
41-50	49	18	20.60%	67
51-60	27	9	10.30%	36
61-70	21	0	0.60%	21
Chi-square = 17.93, df = 4 (P = 0.001); I² = 78% Test for overall effect: Z = 7.37 (P < 0.00001)				

Habits				
Smoking	56	7	0.80%	63
Manipuri	31	6	3.00%	37
Gutka	22	3	4.50%	25
Betel quid	19	2	9.00%	21
Paan	9	8	10.50%	17
non-smokers	23	40	12.00%	63
Multiple habits	43	0	60.20%	43
Chi-square = 104.63, df = 6 (P < 0.00001); I² = 94% Test for overall effect: Z = 9.20 (P < 0.00001)				
Operating Sites:				
Buccal mucosa	50	87	97.80%	131
Tongue	62	0	0.60%	62
Lip	39	0	0.60%	39
Floor of mouth	16	0	0.60%	16
Alveolar region	21	0	0.60%	21
Chi-square = 129.54, df = 4 (P < 0.00001); I² = 97% Test for overall effect: Z = 6.83 (P < 0.00001)				
Gender				
Male	132	79	90.80%	211
Female	50	8	9.20%	58
Chi-square = 15.54, df = 1 (P < 0.0001); I² = 94% Test for overall effect: Z = 7.43 (P < 0.00001)				
Total	182	87	100%	269

The exfoliative cytology diagnostic techniques were employed on 182 oral leukoplakia cases from Group-I and 87 healthy participants from control Group-II. From Group-I about 37.5 % cases of oral leukoplakia were identified as mild dysplasia. Only six (3.3%) cases were severe dysplasia and 28.5 % cases were negative for AgNOR staining (table-II).

Table-II: Degree of Dysplasia following exfoliative cytology (Group-I) n = 182.

Degree of dysplasia	Frequency	Percentage
Hyperkeratosis without dysplasia	19	10.43
Mild dysplasia	68	37.5
Moderate dysplasia	37	20.32
Severe dysplasia	6	3.3
Negative cases	52	28.5

The complexity and negative cases in Group-I following exfoliative cytology were compared by following incisional biopsy method in Group-I patients except severe dysplasia cases 3.3 %. Whereas, comparative sensitivity analysis revealed exfoliative cytology 71.4% sensitive and 93% specific diagnosing oral leukoplakia's (Table-III).

Table-III. Comparative sensitivity and specificity analysis of exfoliative cytology and incisional biopsy histopathology (Chi-square test, 95 % CI).

Diagnostic Technique	Group-I	Group-II	% Risk Ratio	Total	PPV	NPV
Exfoliative test positive	130	6	6.90%	136		
Exfoliative test negative	52	81	93.10%	133		
Total	182	87	100%	269		
Chi-square = 118.60, df = 1 (P < 0.00001); I² = 99% Test for overall effect: Z = 6.66 (P < 0.00001)					95.50%	61%
Sensitivity =	71.40%					
Specificity =	93.10%					

About 176 cases of exfoliative cytology excluding six severe dysplasia cases from Group-I were further assessed by incisional biopsy histopathology using hematoxylin and eosin. The control Group-II participants were analyzed based on only exfoliative cytology due to its convenience against healthy subjects. The histopathological sensitivity of incisional biopsy method was recorded as 81.8%.

DISCUSSION:

Approximately one year research period was expended to test 182 oral leukoplakia cases against exfoliative cytology as well as incisional biopsy histopathology. Our study primary outcomes were based on mitigating existing confusions in research literature related to specificity and sensitivity of either technique that are used to diagnose pre-malignant lesions. Most of the available literature from developing countries enrolled few patients of oral leukoplakia. A research report based on 44 cases, reported that four cases declared negative upon exfoliative examination were found positive on histopathological testing [22]. Whereas in our outcomes 14 cases that were negative for exfoliative cytology were showing mild dysplasia on histopathological analysis of biopsy specimens testing via incisional biopsy histopathological evaluations. Another study led by Hosmani JV et al used exfoliative cytology technique for the diagnosis of pre-malignant lesions and did not confirmed the negative cases after testing with exfoliative cytology by using incisional biopsy histopathology. Where another study led by Svrisky et al. in 2002 reported that 55 negative cases of exfoliative cytology were positive for dysplasia upon histopathological evaluations [23,24]. The

literature search revealed the 61 % sensitivity and 97 % specificity of exfoliative cytology diagnosing leukoplakia as well as well oral squamous cell carcinomas [25]. Whereas our study purely based on leukoplakia cases reported 71.4% sensitivity and 93% specificity of exfoliative cytology as a diagnostic approach. This minimizes existing confusions choosing subjected technique specifically for pre-malignant oral lesions.

CONCLUSION:

Current study based on pre-malignant oral conditions suggest exfoliative cytology 71% sensitive and 93 % specific diagnosing oral leukoplakia. Further, we did not recommend incisional biopsy histopathology for severe dysplasia cases.

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AUTHOR'S CONTRIBUTION:

Waqas Iqbal: Principle investigator, study designing, questionnaire and coordination.
Arhama Surwaich: Incisional biopsies, preservation, and histological evaluations.
Surwaich Ali Channa: Histopathological evaluations and quality assessment.
Abid Hussain Chang: Patient selection.