A systematic scoping review on utility of cytomorphometry in the detection of dysplasia in oral potentially malignant disorders

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ABSTRACT

Background: Oral exfoliative cytology is simple, non-invasive, inexpensive tools prone to subjective bias. Objective computer-based quantitative cytomorphometry technique was developed in last few decades to overcome its limitation. However, there is no consensus about the utility of cytomorphometry as a routine screening tool for early detection of dysplastic changes.

Objectives: To review the utility of cytomorphometry in OPMDs in the detection of dysplasia changes.

Method: A scoping review was undertaken focusing on cytomorphometric analysis in exfoliative cells for detection of oral epithelial dysplasia and cytomorphometric parameter denoting dysplasia. The systematic search was carried out in the online journal databases (Scopus, Google Scholar, PubMed, Web of Science) using keywords until December 2019 for the studies evaluated the cytomorphometry in the detection of dysplastic changes in OPMDs. Three authors independently extracted data using collection forms.

Result: The search strategy found seven analytical cross-sectional studies for scoping review after eliminating 8940 out of 8947 initial results. The scoping review showed that cytomorphometry is useful tool in detecting the dysplastic changes in OPMDs. In addition, decrease in mean cellular area, increase in mean nuclear area, and decrease in mean cell diameter, increase mean nuclear diameter, increase in mean nuclear and cytoplasmic area ratio, associated with development of dysplastic changes in OPMDs.

Conclusion: Cytomorphometry is promising tool, yet there is currently no-good evidence to support role of cytomorphometry in detecting the dysplastic changes in OPMDs in this setting. More research is required for refinement of cytomorphometry as a screening tool.

1. Introduction

Oral Potentially Malignant Disorders (OPMDs) are group of disorders; with malignant transformation rate varies between OPMD and ranges from 1.4% to 7%.^{1,2} One of the predictive parameters for risk of malignant transformation in all OPMDs is the epithelial dysplasia.³ It has become paramount importance to detect the epithelial dysplasia in OPMDs for early detection and intervention of malignancy. Histopathological examination of invasive tissue biopsy can diagnose dysplastic change prequel to oral cancer. However, it has several limitations such as technical expertise, incisional biopsy, experienced

pathologist, tissue processing facilities, tissue processing time, inter-observer and intra-observer variation.⁴ In past, various non-invasive tool i.e. Fluorescence imaging, Optical spectroscopy, Vital staining, Photodynamic detection, Chemiluminescent kit, Confocal Microscopy, Optical Coherence Tomography, etc. were used to detect the early dysplastic change within epithelium in OPMD's.⁵ These techniques have demonstrated positive results, but can be strictly used as clinical adjunct, not as diagnostic tool.⁶ Further, these tools cannot tell about the cellular and nuclear alterations in the mucosa, which is important for diagnosis of lesion and hence, they have a limited utility in early detection of OSCC as a diagnostic tool. In contrast, exfoliative cytology is

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Fig. 1. Flow diagram of the study selection process (PRISMA Flow diagram).

a non-invasive method, which assist in detection of alterations occurring in cell morphology (cellular and nuclear) through microscope.^{7–9} In 2017, World Health Organization (WHO) recommended the numerous criteria such as abnormal variation in nuclear and cell size, increase in Nuclear: Cytoplasmic ratio, etc. for characterization of dysplasia. However, analysis of these factors are more or less subjective and no objective method mentioned to weigh them [WHO 2017 criteria].¹⁰ Cowpe et al. was one of the early researcher to explore the role of quantitative morphometric analysis of cytology smears for nuclear and cytological parameter and to reduce subjective variation.¹¹ These parameters were found to be of great value for monitoring and follow-up of suspicious lesion for risk of malignant transformation and early detection of OSCC. However, there is scarcity of studies, which has tried to quantify the morphometric parameter (nuclear and cytological) of cells in normal and OPMDs of oral cavity.

Cytomorphometry need to be explored as screening tool for detection of dysplastic changes in OPMDs. We hypothesized that increase nuclear, decrease cellular area with altered/increase nuclear-cell area ratio in cytology of OPMD's is indicative of dysplastic changes. With this view in mind, we formulated the objectives such as (1) to explore whether cytomorphometry (Intervention) can detect the dysplastic changes (Increase Nuclear Area (NA), decrease cytoplasmic Area (CA), altered NA/CA ratio change among cells) (Outcome) in OPMDs (Participant) and (2) to construct cytomorphometric cut-off value for discriminating between OPMDs as non-dysplastic or dysplastic which can be utilized for future research. This scoping review aimed to identify and map evidence on utility of cytomorphometry in detection of dysplastic changes in OPMDs. The outcomes of the scoping review

was used to inform whether cytomorphometry can be utilized, as screening tool for early identification.

2. Methods

The scoping review is in agreement with the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Statement.¹² The ethical committee approval was not necessary to conduct scoping review because it was conducted on data available in the public domain from previous published studies.

2.1. Study scope and definition of the index standard, research question

The scoping review research question: Is cytomorphometry useful for detecting dysplasia (quantitative cellular and nuclear morphological) changes in OPMDs. For the purpose scoping review, presence of dysplasia on cytomorphometry analysis is defined as decrease in cell area, increase in nuclear area, and increase in nuclear-cytoplasmic ratio.

2.2. PCC (population, concept, context)

The PCC framework for these questions was as follows:

Population – All age and gender, Patients diagnosed/suspected of having oral potentially malignant disorders.

Concept- Cytomorphometry can identify the morphological changes, in exfoliative cells collected from OPMD's, akin to identification or detection of dysplasia.

Table 1
Characteristic of studies included for scoping review of cytormorphometry in oral premalignant disease and oral squamous cell carcinoma.

Study Author(s)	Verma et al.	Shaila et al.	Ramaesh et al.	Mollaoglu et al.	Kuyama et al.	Bhavasara et al.	Archana et al.
Year	2015	2016	1998	2001	2013	2011	2018
Country	India	India	Shri Lanka	Turkey	Japan	India	India
Sample size (n)	90	105	176	109	60	300	40
Intervention group	60	52	136	33	40	200	30
	OLD (n = 30)	Leukoplakia with atypia (n = 18)	No epithelial dysplasia (n = 58)	SCC (n = 33)	Dysplasia (n = 30)	Oral leukoplakia (n = 45)	Oral leukoplakia (n = 10)
	OSCC(n = 30)	Leukoplakia without atypia (n = 34)	Epithelial dysplasia (n = 27)		OSCC (n = 10)	Oral lichen planus (n = 10)	Oral submucous fibrosis (n = 10)
			OSCC (n = 51)			Oral submucous fibrosis (n = 45)	OSCC (n = 10)
Case selection	Histopathological confirmed cases	Cytologically confirmed cases	Histopathological confirmed cases	Histopathological confirmed cases	Histopathological confirmed cases	OSCC (n = 100)	Histopathological confirmed cases
Control group	30	53	40	76	20	100	10
Control Site	Healthy oral mucosa	Healthy oral mucosa	Buccal mucosa	Floor of mouth	Normal oral mucosa	Normal oral mucosa	Normal oral mucosa
Age	Above 49 years	–	Above 40 year	39–98 years (mean age 64.18 years)	–	Male (mean) – Female (mean) – 55.36 ± 21.27 52.3 ± 11.84	–
Gender	Male = 54 Female = 36	Male = 98 Female = 12	Male = NA Female = NA	Male = 09 Female = 24	Male = NA Female = NA	Male-195 Female-105	Male = NA Female = NA
Standard Test Intervention/	Histopathology	Histopathology	Histopathology	Histopathology	Histopathology	Histopathology	Histopathology
diagnostic test	Cytomorphometry	Cytomorphometry	Cytomorphometry	Cytomorphometry	Cytomorphometry	Cytomorphometry	Cytomorphometry
Cytology	Cytobrush	Wooden spatula	Wooden spatula	Cytobrush	Cytobrush	Cytobrush	Wooden spatula
Cytology stain	Modified Rapid PAP stain (Bio-lab Diagnostics)	PAP stain	PAP stain	PAP stain (Ortho Diagnostics)	PAP stain	Rapid PAP kit (Sigma Aldrich Diagnostic)	H & E stain
Cytomorphometry technique	Image analysis (Imagepro Express 6.0 Media Cybernetics)	Motic image analyzing software, 2003, version 2.0)	Calibrated eyepiece micrometer	Seescan TV image analysis software	Cell measurement software (Win Roof version 2.5, Mitani Co.)	Calibrated eyepiece micrometer	Calibrated eyepiece micrometer
	Automatic method	Automatic method	X100 objective	X40 objective	Automatic method, 40x objective	X100 objective	X100 objective
	X40 Objective	X40 objective	Direct method			Direct method	Direct method
Number cells counted per slide	125	50	100	50	100 to 150 cell in each group	100	100
Parameter studied	NA, CA, NA/CA ratio	CD, ND, CA/NA, NA/CA ratio	CD, ND and ND:CD ratio	CA, NA	NA, ND, CA, CD	CD, ND and ND/CD ratio	CD, ND, and ND/CD ratio
Primary out come	Increase in NA (p < 0.001) Decrease in CA (p < 0.001)	Progressive increase in CD and CA from group 1 to group 3 (p < 0.05) Decrease in ND and NA from group 1 to group 3 (p < 0.05) No significant difference in NA:CA ratio between Group 1, 2 and group 3	Decrease in CD from normal to SCC and increase in ND from normal to SCC	Significant increase in NA (p < 0.01) and reduction in CA (p < 0.001)	NA & ND shows reduction in dysplasia (mild & moderate) and increase in severe dysplasia & OSSC. (p < 0.05) as compared to normal mucosa Increase in NA:CA ratio from normal to OSSC (p < 0.05)	Increase in ND (p < 0.0001) and decrease in CD from normal to OSSC (p < 0.0001) Increase in ND:CD ratio in dysplasia and OSSC (p < 0.0001) as compared to normal.	Reduction CD and increase in ND in study group as compared to control group (p < 0.0001) Increase in ND/CD ratio study group as compared to control group (p < 0.0001)
Conclusion	Useful Diagnostic adjunct in early detection of oral cancer	Useful Diagnostic tool in early detection and screening of oral cancer	Sensitive parameter in diagnosis of oral premalignant and malignant lesion	Additional diagnostic test for monitoring and detecting oral malignancy in early stage	Useful Diagnostic adjunct in early detection of oral cancer	Detect early precancerous changes.	Useful Diagnostic adjunct in early detection of oral cancer

*Cytomorphological change indicative of dysplasia or malignancy; OSSC: Oral squamous cell carcinoma; NA: nuclear area; CA: cytoplasmic areas; ND: Nuclear diameter; CD: cytoplasmic diameter; PAP: Papanicolaou stain.

Table 2
Cytomorphometric data of included studies.

Year	Author's	Clinical diagnosis	Histopathological diagnosis	Cases/ Samples	Cell diameter	Cell area	Nuclear Diameter	Nuclear Area	NA:CA ratio	ND:CD ratio
1998	Ramaesh et al. ¹⁹	Normal Mucosa	Normal	58	51.78 ± 0.11		8.36 ± 0.49			
		Lesion with dysplasia	Dysplasia	27	41.32 ± 0.13		9.04 ± 0.46			
		OSCC	OSCC	51	38.58 ± 0.11		10.10 ± 0.56			
2001	Mollaoglu et al. ²⁰	OSCC- Tumor	OSCC	37		1784 ± 500		99.5 ± 29.99		
		Normal- Contralateral	Normal	30		2457 ± 560		89.0 ± 13.11		
		Normal (No lesion)	Normal	46		2516 ± 408		84.5 ± 10.3		
2011	Bhavasari et al. ¹⁵	Normal Mucosa	Normal	106	83.6 ± 6.72		16.8 ± 2.13			0.2608
		Lesions with mild to moderate dysplasia	Mild to moderate dysplasia	57	77.9 ± 7.66		17.2 ± 3.60			0.2207 ± 0.04783
		Lesions with severe dysplasia and carcinoma in situ	Severe dysplasia and carcinoma in situ	30	60.2 ± 11.12		18.3 ± 2.96			0.3039 ± 0.10116
2013	Kuyama et al. ¹⁶	Oral Squamous cell carcinoma	Oral Squamous cell carcinoma	107						
		Normal Mucosa	Normal	20	51.5 ± 9.3	10404.2 ± 4429.9	20.3 ± 3.60	222.2 ± 99.8	0.02	0.10758
		Mild dysplasia	Mild dysplasia	10	109.7 ± 35.0	2099.2 ± 1127.0	12.2 ± 2.5	199.7 ± 59.0	0.1	
		Moderate dysplasia	Moderate dysplasia	10	50.1 ± 13.1	1978.1 ± 1517	15.7 ± 2.4	201.3 ± 59.0	0.1	
		Severe dysplasia	Severe dysplasia	10	48.7 ± 30.0	746.0 ± 421.9	15.9 ± 3.6	251.7 ± 95.3	0.34	
		OSCC	OSCC	10	28.9 ± 7.7	1490.8 ± 1252.9	17.4 ± 5.2	592.9 ± 461.8	0.4	
2015	Verma et al. ¹⁸	Oral leukoplakia with dysplasia	Dysplasia	30	40.6 ± 15.8	1078.51 ± 56.65	25.8 ± 9.5	107.97 ± 5.44	0.100 ± 0.008	
		OSCC	OSCC	30		769.70 ± 38.77		139.02 ± 8.10	0.181 ± 0.015	
		Normal Mucosa	Normal	30		1535.80 ± 79.38		65.47 ± 4.77	0.043 ± 0.004	
2016	Shaila et al. ¹⁷	Leukoplakia cases with atypia	Dysplasia	18	48.24 ± 3.68	11371 ± 1716	9.48 ± 1.04	331.2 ± 41.1	0.03032 ± 0.00467	
		Leukoplakia cases without atypia	No dysplasia	34	53.39 ± 4.96	11803 ± 1619	9.372 ± 0.867	303.7 ± 34.0	0.02684 ± 0.00462	
		Normal controls.	Normal	53	51.68 ± 4.04	11877 ± 1196	8.841 ± 0.557	248 ± 17.6	0.02160 ± 0.00257	
2018	Archana et al. ¹⁴	Oral leukoplakia	No dysplasia	10	31.66 ± 16.53		9.05 ± 1.92			0.33 ± 0.11
		OSMF	No dysplasia	10	28.32 ± 9.38		9.17 ± 2.09			0.35 ± 0.11
		OSCC	OSCC	10	19.11 ± 3.27		10.69 ± 2.27			0.58 ± 0.17
		Control	Normal	10	42.04 ± 5.56		5.57 ± 0.76			0.13 ± 0.02

Context: Publication investigating or evaluating or analyzing cytomorphometry in OPMDs and compared it to normal mucosa, OSCC, or both to identify or diagnose OPMDs.

2.3. Eligibility criteria

The inclusion criteria were: (1) cross-sectional/cohort/case-control and retrospective/prospective study that performed cytomorphometry in exfoliative cytology for detection of dysplastic changes in OPMDs; (2) compared either with normal mucosa or malignant mucosa; (3) measured or reported cellular and nuclear parameters i.e. CA, NA and NA:CA ratio and/or CD, ND, ND:CD ratio as outcome; (4) studies that included cases diagnosed as OPMD on histopathology, cytopathology or clinically (later confirmed on histopathology); (5) studies that used PAP or H & E (routine stain); (6) studies used cytobrush or wooden spatula for collection of cells. Only original research articles published in English language, with no restriction on the year for assessment were included in the present scoping review. The expected outcomes anticipated in included studies for the present scoping review were increase Nuclear Area (NA), decrease Cytoplasmic Area (CA), increased NA/CA ratio among exfoliative cells by cytomorphometry in OPMDs either reported as 2×2 contingency table or as measurements with mean, Standard Deviation (SD) or Standard error (SE).

2.4. Information sources

Scopus, Google Scholar, PubMed and, Web of Science database were searched until December 2019. The results of search string used and output obtained has been mentioned in Fig. 1. Review articles, short communications, correspondence, editorials, letter to the editor etc. were removed from consideration. Moreover, grey literature was also searched for obtaining the articles relevant for the scoping review. Cross-references from individual papers were also considered for retrieving the data.

2.5. Search strategy

Two reviewers developed the search strategies with the help of librarian (SP). In view of scoping review, search was iterative in nature to identify all relevant and potential studies. The search strategy included the use of keywords and relevant indexing to identify articles related to research question(s) in first step. In second step, analysis of abstract and title for additional keywords for further search in online databases. Third step involved identification of additional studies within identified articles and references list. The following keywords used were “Oral premalignant disease”, “Oral premalignant lesion”, “Oral submucous fibrosis”, “oral lichen planus”, “oral leukoplakia”, “leukoplakia”, “cytomorphometry”, “cytology”, and “morphometry”.

2.6. Study selection

Three reviewers (MY, AG, SG) have independently screened the retrieved titles after duplicate articles were removed. Titles and abstracts were screened for determination of eligibility of articles for inclusion. Final selection was carried based on full-text reading by two reviewers (MY, AG) according to pre-defined inclusion and exclusion criteria. Any differences regarding eligibility, inclusion or data interpretation was resolved through discussion and arbitration by a third reviewer (SS).

2.7. Data item and collection

A charting table was created to record characteristics of included studies and key information to scoping review question. Data from the eligible studies after full-text screening was extracted independently by three reviewers (MY, ST and SM) using a pre-defined charting table.

Extracted data included details of study (author/s, year of publication, country), details of participants (sample size, type of lesion, site of lesion, age and gender distribution of participants), cytomorphometry (techniques, cytological stain used, cytomorphological parameters, and morphometric values of cells studied [mean and standard deviation (SD)]), histopathology diagnosis (standard reference test), outcome of study, key findings related to the scoping review questions. The attempted was made to contact authors for further information when data to construct was unclear or additional data were required.

2.8. Synthesis of results

A descriptive numerical summary of the characteristics of the included studies was performed. Tables were created to reflect the overall number of studies included, study designs and settings, publication years, the characteristics of the study populations, collection tool and methods used, cytology stain, reference/gold standard test, cytomorphometry parameter measured, the outcomes reported, and the countries where the studies were conducted (Table 1 and Table 2). As outcomes of interest were continuous data, the means and standard deviations for cytomorphometry parameter in normal mucosa, OPMDs and OSCC were collected to obtain answer of research question proposed.

2.9. Critical appraisal of included studies

To address the research question, at two reviewers (MY, AG) independently assessed the quality of included studies with Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Analytical Cross Sectional Studies and summary of it was generated.¹³ Each study was rated either ‘Yes’, ‘No’, ‘Unclear’ or ‘Not Applicable’ Disagreements were resolved by consensus or by involving a third reviewer (SG).

3. Results

3.1. Study selection

The screening details are presented in a PRISMA flow diagram (Fig. 1). The search strategy yielded 8947 results. During initial evaluation of titles and abstracts, total 8917 articles were excluded from the analysis based inclusion and exclusion criteria. Out of 30 shortlisted articles, seven articles were finally selected for the present analysis.

3.2. Characteristics of sources of evidence

Seven cross sectional studies were published from 2015 to 2018.^{14–20} Five were from India and 1 each from Srilanka, Turkey, Japan. Analytical cross section study ($n = 6$) was most common type of study design. Six Studies compared OPMDs with either normal mucosa and/or OSCC where as one study compared between OPMD and normal mucosa. In all, total 844 participants (356 males; 448 females) were investigated. Gender distribution was not mentioned in the studies by Ramaesh et al.,¹⁹ Kuyama et al.¹⁶ and Archana et al.¹⁴ Among the OPMDs, leukoplakia ($n = 222$) was the most common lesion followed by oral submucous fibrosis ($n = 55$) and oral lichen planus ($n = 10$). OSCC ($n = 234$) was utilized for comparing the observation (positive control-malignancy) where as oral mucosa ($n = 317$) (negative control-No malignancy/no dysplasia). Age of 451 participants were 26 years or above while Kuyama et al.,¹⁶ Archana et al.,¹⁴ Shaila et al.¹⁷ did not mention age of the participants. Histopathology ($n = 6$) was the most common gold standard utilized for confirmation as well as selection of participants. The OPMDs diagnosis was done on histopathology in majority studies. Exfoliative Cytology and cytomorphometry was utilized in all studies and PAP was most commonly used histology stain. Four studies used cytobrush whereas three studies used wooden spatula to collect exfoliative cell from the site. Three studies used automated

morphometry software while four studies used manual calibrated micrometer eyepiece. All studies either used 40x or 100x to measure parameter. Minimum 50 cell per slide (range 50–150 cells) were evaluated. Additional characteristics of these seven studies are summarized in Table 1.

3.3. Critical appraisal of included studies

Seven studies for the scoping review were appraised against the Joanna Briggs Institute Critical Appraisal tools for analytical cross-sectional studies to determine the quality of included studies. Not all studies met all criteria (Supplementary Table S1). The included studies have not taken into account, age and gender variation. No study compared the cumulative effect of tobacco chewing duration and frequency on morphology of cells studied. All the 7 studies^{14–20} did not mention the number of true positive and false negative cases. 6 studies^{14–16,18–20} did not specify exact nature of clinical lesion or clinical type of leukoplakia except study conducted by Shaila et al.¹⁷ Additionally, not all OPMD, tobacco related oral mucosal lesion, OSCC stages (if any) were classified in the same way, and not all the studies specified if such lesions were reported by a standardized (or always the same) examiner to remove observer bias. Only three studies (Bhavasara et al., 2011; Kuyama et al., 2013; Ramaesh et al., 1998)^{15,16,19} have used WHO criteria for oral epithelial dysplasia (mild, moderate, severe). Two studies (Bhavasara et al., 2011; Shaila et al., 2016)^{15,17} recorded tobacco/areca nut chewing habit among participant. Although two studies (Kuyama et al., 2013; Shaila et al., 2016)^{16,17} performed cytology confirmation of presence of epithelial dysplasia by two or more cytologists/pathologist, no studies including these two studies have conducted kappa analysis to find degree of agreement.

3.4. Cytomorphometry in detecting dysplastic changes in OPMDs

Seven studies reported changes in cellular and nuclear parameter of cells in OPMDs as compared to normal mucosal cells or malignant cells or both. Three studies (Verma et al., 2015; Ramesh et al., 1998; Bhavasara et al., 2011)^{15,18,19} used histopathology as gold standard where as one study (Shaila et al., 2016)¹⁷ used cytology for diagnosis of dysplasia using double blinding technique to avoid bias. To answer research question, cytomorphometry values (mean and SD) of exfoliative cytology of normal mucosa, OPMD's and OSCC from individual studies was extracted (Table 2). The cytomorphometric data of seven studies on detection of epithelial dysplasia (Based on quantitative morphometric data) showed decrease CA and CD, increase NA and ND, increase in NA: CA and ND: CD ratio values during transition normal mucosa to OPMD's with epithelial dysplasia to malignancy. This indicates that cytomorphometry analysis of exfoliative cytology can detect dysplastic changes.

4. Discussion

Cytomorphometry is an objective quantitative tool, which was studied to assess its diagnostic capability and had demonstrated promising role in detection of dysplasia in OPMD's. The studies observed the alteration in cellular and nuclear morphology in exfoliative cells from OPMD's as compared to normal mucosa or OSCC is indicative of dysplastic changes. However, smaller sample size and absence adequate randomization may limit appropriate interpretation of study outcome.

To the best of our knowledge, this is the first scoping review to find out the role of cytomorphometry in detection of dysplasia in OPMDs. The scoping review of cytomorphometry as screening tool was warranted for following reasons: assessment of utility of cytomorphometry as screening tool in the clinical setup, evaluate quality of primary analytical studies, obtain information to suggested further development of screening tool.

We found that cytomorphometry could detect the

cytomorphometric changes indicative of dysplasia in exfoliative cytology of OPMD's. All seven included studies have compared the cytomorphometry against the histopathology (Gold standard) or cytopathology. Although, overall critical appraisal was low in included studies, absence of reporting of sensitivity and specificity in form of false positive and false negative cases may underscore the conclusion. Well-designed cohort studies with large sample is essential to further development in cytomorphometry as screening tool.

The present scoping review observed that cytomorphometry could differentiate nuclear and cytological change in OPMD's with epithelial dysplasia using quantitative measurements of cells in exfoliative cytology smears. Cellular parameters undergo variations as dysplastic changes initiates in normal mucosa. CD and CA was decreased in OPMD's with epithelial dysplasia and OSCC. It is common understanding that during proliferation cell size gets double with duplication of cell organelles.²¹ Possible mechanism behind it is poorly understood and need to be explored. There may be different possible mechanism for epithelial proliferative activity in initial stage of development of malignancy such as alteration in certain transcriptional regulator and cell cycle regulator.²² In physiological cell proliferation, cell cycle and cell growth process are in coordination with each other but they may a separate process in malignancy.²³ That may explain the reduction in cell size (both area and diameter) in case of epithelial dysplasia and OSCC. The scoping review did not show any significant difference which was similar to that of Cowpe et al.¹¹ and Franklin et al.²⁴

Dysplastic/malignant cells usually show increase in nuclear size, sometimes with irregular shape.²⁵ Increase in nuclear size could be due increased in synthesis of DNA.²⁶ It was observed that abnormal DNA content increases from mild to severe degree of dysplasia²⁷ and has been suggested as predictor of oral epithelial dysplasia.^{27,28} This scoping review observed that OPMD's with epithelial dysplasia have higher ND and NA as compared to normal mucosa. Similarly, epithelial cells from tobacco/areca nut chewers have shown increase in ND and NA.^{29,30} The change in ND and NA could be due to cellular adaptation needed for cell survival, proliferation, invasion and migration.³¹ Although nuclear morphometry is good indicator of dysplastic change, it should be interpreted cautiously due to reason reported in previous studies.³² This aspect needs further exploration with studies involving the hyperplastic inflammatory lesion.³³ Nuclear morphometry is an effective objective parameter in case of borderline cases having dilemma.³⁴

Each mammalian cell has characteristic nucleus to cytoplasm ratio and it plays important role in natural development, any change ratio can deviate it to pathology.³⁵ Cytopathologist takes the NA: CA ratio as important features of dysplastic changes. Usually NA: CA ratio involves the ratio of nuclear area and cell area, not the cell and nucleus diameter. The few cytology reporting systems have provided the well-defined NA:CA ratio cut-off value for each (dysplasia) grade.³⁶ However, there is no cut-off value for oral epithelial dysplasia on cytology. It is observed the cytomorphometry can detect increase NA:CA ratio, but, it can be interpreted differently.³⁷ Currently, there are no studies determining the cut-off value for NA: CA ratio for dysplasia in oral region as well as evaluating the accuracy of oral pathologist/cytopathologist. Hang JF et al. suggested NA:CA ratio cut off value of ≥ 0.5 for high grade carcinoma based on digital analysis of smears.³⁸ In included studies, NA: CA ratio range was from 0.1 to 0.4, however, it need a through investigation before standardizing it as cut-off value of dysplasia in oral exfoliative cytology.

Oral Exfoliative cytology is preferred non-invasive method, routinely employed at clinics for initial screening of oral mucosa lesion for detection malignant changes.^{39,40} It gives advantage to oral pathologist/clinician to inform patient about the presence of malignant changes and helps in clinical management of oral lesions. There is correlation between clinical features and diagnosis of dysplasia/malignancy in case of OSCC.⁴¹ However, correlating clinical features with diagnosis of dysplasia on exfoliative cytology are scarce. Current studies

in scoping review have not mentioned or studied the correlation between degrees of dysplasia with clinical appearance of lesion, although clinical diagnosis was mentioned. It is difficult to diagnose OPMD's with dysplasia on clinical appearance where exfoliative cytology comes in handy. This simple technique requires minimal tools to collect the cells, which can be examined with 20–30 min to give diagnostic impression. In case, OSCC diagnosis on exfoliative cytology do not have any difficulties considering clear definitive features, however, it is not true in case of OPMD's with dysplasia. Furthermore, there is no well-developed and authenticated grading system for oral epithelial dysplasia due to lack of follow-up studies and insignificant correlation between grade of dysplasia and malignant transformation rates (5% in mild, 3–15% in moderate and 7–50% in severe/Carcinoma in-situ).⁴² In scoping review, no other study except Shaila et al., has mentioned about either presence/absence of dysplasia or degree of dysplasia in smears.¹⁷

Bethesda system⁴³ is routinely used for grading in cervical pathology since 1988; however, oral pathologist has been utilizing the PAP test and classification for smear interpretation. Since oral pathologist generally uses PAP stain, it understandable to follow PAP smear interpretation technique by oral pathologist. Most of studies in scoping review used PAP stain followed by hematoxylin-eosin stain. As not all of these stains are known for producing appropriate nuclear and cytoplasmic visualization, staining related variations in interpretation is expected. PAP classification range from absence of atypical or abnormal cells to conclusive for malignancy.⁴⁴ However, lacks mentioning of (degree of) dysplasia in classification similar to Bethesda system, which is important from clinical management aspect. Oral exfoliative cytology studies mention about the presence of dysplasia but usually lacks to mention about the grade and grading system.^{9,17} Similar observations were found in scoping review where studies did not mention about dysplasia grade in smears. This could be due to system used for reporting the cytology smears. Further, these cytomorphometric studies were retrospective and lesions were diagnosed as dysplastic, histopathology examination instead of exfoliative cytology. Dysplasia grading on tissue histopathology is well established and is easy to perform as World Health Organization (WHO) classification for epithelial dysplasia is well documented and it may show inter-observer and intra-observer agreement variation.⁴⁵ However, no such criteria are established for oral exfoliative cytology.

In 2013, the Japanese Society of Clinical Cytology (JSCC) developed guideline for oral cytology based on Bethesda system. Sekine et al. studied the diagnostic applicability of exfoliative cytology in dysplasia based on Bethesda system.⁴⁶ Their results indicated Bethesda system can diagnose histologically negative (non-malignant lesions including inflammatory); OIN/CIS (oral intraepithelial neoplasia or carcinoma in situ); and positive (squamous cell carcinoma and other malignancies) but Borderline lesion -Negative (mild, mild-moderate, or moderate dysplasia); Borderline lesion-positive (severe dysplasia) can cause difficulty. Hence, they recommended cytological diagnoses of LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), and SCC (squamous cell carcinoma) should be examined microscopically. However, Bethesda system is also prone for interobserver variability⁴⁷ with variable kappa agreement.^{48–50} Usually, studies evaluating oral cytology do not perform smear interpretation by two or more cytopathologist or report interobserver agreement or kappa analysis. Similarly, Shaila et al. has performed double blind smear interpretation involving two different cytopathologist without kappa analysis.¹⁷ Hence, it is difficult to comment on interobserver agreement for PAP or WHO system used for interpretation of cytology.

The included studies in scoping review has certain gaps and lacunae. Sensitivity and specificity, or alternatively positive predictive value (PPV) and negative predictive value (NPV) for cytomorphometry were not reported. Similarly, included studies did not mentioned about number of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) cases. Other features of dysplasia^{2,51} like

hyperchromatism, nuclear and cellular pleomorphism, abnormal mitosis etc. that could also determine the malignant potential of OPMD are not considered in all the papers. Confounding variables such as age, gender, site of the lesions, etiological factors, duration and frequency of tobacco chewing or smoking habits are the major limitations of the present study as they can directly or indirectly related to the malignant transformation.^{52,53}

5. Conclusion

This scoping review indicate that there is currently no-good evidence to support role of cytomorphometry as screening tool for early detection of dysplastic change in oral premalignant disorders as well as malignancy conversion of OPMD's. Availability of morphometric cutoff value to denote conversion into dysplasia/malignancy could bring more objectivity to it. The future research should focus on finding the morphometric (quantitative) cut off value for denoting dysplastic epithelial changes and early changes of malignant transformation by using standardized methodology in large cohort at multi-centric. Also standardization of cytomorphometry analysis method may improve detection capacity of pathologist, which eventually helps clinician in making decision in case of treatment dilemma.

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Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jobcr.2020.06.016>.

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