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UTILITY OF BRUSH CYTOLOGY IN EVALUATION OF PRE-MALIGNANT AND MALIGNANT ORAL MUCOSAL LESIONS AMONG DENTAL PATIENTS ATTENDING KENYATTA NATIONAL HOSPITAL
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J.A. Ojwang, W. Waweru, E. Walong, M.N. Kanini and R. Chibvongodtze

ABSTRACT

Objectives: **The main objective was to determine the utility of brush cytology in evaluation of pre-malignant and malignant oral mucosal lesions.**

Methodology: **This was a prospective cross sectional descriptive study which was carried out at Kenyatta National Hospital (KNH), Dental clinic, Surgery unit and University of Nairobi, Dental Hospital (UoN DH) from November, 2015 to April, 2016 after obtaining ethical clearance from Kenyatta National Hospital – University of Nairobi Ethics and Research Committee (KNH-UoN ERC) on participants who presented with pre malignant and malignant oral lesions. Participation in the study was voluntary and an informed consent was obtained from all participants. A structured questionnaire was used to collect socio-demographic information and clinical history. A cervical cytobrush brush (Andwin Scientific – Woodland Hills, CA 91303 USA) was used to sample oral mucosal lesions and later biopsy performed for histopathology. Oral brush cytology samples were fixed in 95% ethanol, cytospined and stained with Pap stain. Data was entered and analyzed using SPSS v 22. The results were presented using tables, charts and disseminated through presentations in conferences and publication in peer reviewed journals.**

Results: **A total of 47 cytology and histology specimens were taken during the study and all samples were satisfactory for evaluation. The female to male ratio of participants was 1: 1.2 with an age range of 25-79 years and mean of 55 years. The main cytological patterns established at KNH and UoN Dental School Hospital was HSIL and SCC representing 97% of dysplastic and malignant lesions. Histology confirmed dysplasia and malignancy in 28 of 30 cytologically diagnosed cases. There were 2 false positives and 1 false negative cases reported. The sensitivity, specificity, positive predictive value and negative predictive values were 97%, 89%, 93% and 94% respectively with a substantial diagnostic agreement (kappa value) between cytopathology and histopathology of 86% and a p-value of ≤ 0.001.**

Conclusion: **Brush cytology has been shown to be a reliable cytological technique for screening and early detection of oral mucosal abnormalities as it has a high sensitivity and a substantial diagnostic agreement with histopathology.**

INTRODUCTION

Oral mucosal lesions are any changes in the oral mucosa that may present as red, white, ulcerative, pigmented or hyperplastic. More than 27% of the patients visiting Dental Clinics present with one or more oral lesion(s) (1). Oral mucosal malignancies are among major causes of morbidity and mortality worldwide. Globally head and neck cancer is the sixth most common cancer of which 40% are squamous cell carcinoma (SCC) (2). In Kenya the prevalence of oral cancer is about 3.6% of all malignancies with minimal annual variation as reported by Onyango et al (2004) and oral squamous cell carcinoma (OSCC) is the most common (3).

Epithelial dysplasia is an indicator of malignant development. Majority of oral lesions such as leukoplakia, erythroplakia, lichen planus and actinic keratosis are considered to be pre-malignant for oral squamous cell carcinoma since a risk of malignant transformation is associated with them. They are often asymptomatic and therefore require regular monitoring particularly among persons who use tobacco products or alcohol (4).

Despite oral mucosa being easily accessible for direct visual examination, malignancies of the buccal mucosa, soft palate, floor of the mouth, hard palate, dorsal tongue and gingival area are often diagnosed when advanced resulting in poor survival rates (5). Exfoliative cytology is a diagnostic technique that can be useful in early diagnosis of oral mucosal pre-malignant and malignant lesions. Majority of oral malignancies are squamous cell carcinomas, adenocarcinomas, adenosquamous carcinomas and malignant melanomas for which early diagnosis using exfoliative cytology is feasible (6,7). Oral brush cytology is a safe, simple, non-invasive, rapid and cost-effective technique in which cells are collected over a wide area and can be implemented easily in resource constrained areas (6). This technique was first used by Morison et al in 1949 when he successfully identified malignant cells in the oral mucosa using exfoliative cytology. We performed a cross-sectional descriptive study at KNH - Dental Clinic Surgery unit and University of Nairobi Dental School Hospital and evaluated the utility of a cheap and rapid brush cytology technique for screening and diagnosis of oral premalignant and malignant lesions.

MATERIALS AND METHODS

This was a prospective cross sectional descriptive study. Recruitment of study participants and specimen collection was done at KNH-Dental Clinic in the Oral surgery section and UoN-Dental Hospital from Patient's aged 18 years and above presenting with clinically confirmed oral lesion(s) suspected to be pre-malignant or malignant at KNH-Dental Clinic and UoN-Dental Hospital. Non probability purposive sampling method was used for this study.

Oral brush specimen for cytopathology was collected by a Dental surgeon before the diagnostic biopsy procedure. The lesion was visualized under adequate illumination. Local anesthesia was administered and the area around the lesion cleaned with normal saline. A commercially available sterile hard nylon cervical cytobrush (Andwin Scientific – Woodland Hills, CA 91303 USA) was used to obtain oral mucosal cells with minimal discomfort by brushing repeatedly in the same direction over the entire lesion until pinpoint bleeding was obtained signaling entry into lamina propria thus obtaining epithelial cells through the full thickness of

the epithelium.Material obtained on the brush was fixed immediately in 95% ethanol by immersion of the brush into the vial. Vortexing was done and the contents in the vials transferred to cytofunnel for spinning in a manual Shandon ® papspin ™ centrifuge and eventual deposition of cells onto the clean charged microscope slides.

The smears were stained using Papanicolaou staining method which is the best staining method for alcohol fixed smears.It gives excellent nuclear and cytoplasm details. All SOP's for these staining methods was followed to ensure and increase reliability and reproducibility of the results (8). The specimen adequacy was assessed microscopically and those slides with at least 30 well preserved parabasal, intermediate or superficial cells, not obscured by blood, exudates or necrosis were considered adequate and analyzed (9).

All the samples were examined by the principal investigator and signed out by two Consultant Pathologists. Presence or absence of cytological findings both epithelial and non-epithelial was reported based on the modified Bethesda Reporting System (TBS) protocol (10). Discrepancies in cytology were reported by an independent Pathologist. The biopsies were obtained, processed, stained and interpreted by two consultant pathologists independent of cytology report. The grading of dysplasia and squamous cell carcinoma was done based on the squamous intraepithelial neoplasia classification system adopted by WHO for reporting (11). Discrepant results were given to a third Pathologist as a tie breaker. These results later were compared with brush cytology results. KNH / UoN Histopathology laboratory guidelines and SOP's were followed.

Clearance and approval was obtained from Kenyatta National Hospital (KNH) and University of Nairobi (UoN) Ethics and Research committee certificate number P531/08/2015 before commencement of the study. Permission to research in the KNH Dental Clinic and UoN Dental School Hospital was sought from the Assistant Director Dental Services and Chairman, Oral Maxillofacial, Medicine and Pathology Department (OMMPD) respectively. Informed consent was obtained from all potential participants using a consent form. Brush cytology procedure was done before biopsy and the local anesthesia was administered to reduce the pain. Patients' privacy and confidentiality was observed.

RESULTS

A total of 47 cytopathology and histopathology specimens were taken during the study period from 1st December, 2015 to April, 2016. The samples were collected from 47 patients in KNH Dental Clinic Surgery Unit and UoN Dental School Hospital who met the inclusion criteria and accepted to participate by signing an informed consent form. All the 47 cytology and histology samples were satisfactory forming the basis for the current data analysis. Majority of the participants were of male gender that is 26 and 21 female representing a 55% and 45% respectively with a ratio of 1.2:1 for M: F (Figure 1). The age range of participants was from 25-79 years old with a mean of 55 years. Majority of the participants of both gender were 51 years and above representing 60% of the total participants as shown in table 1.

Figure 1
Percentages of participants by gender

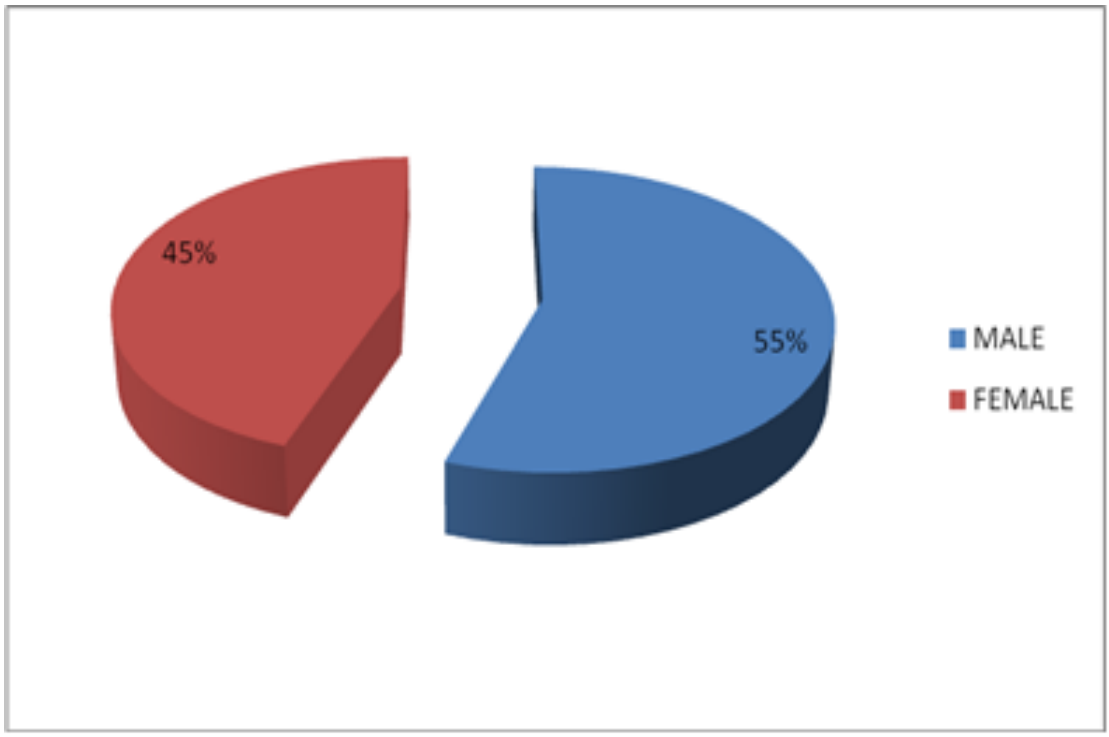


Table 1
Gender vs. age distribution of participants

Gender of participants			Age distribution of participants					Total
			18-28	29-39	40-50	51-61	62 and above	
	Male	Participants count	0	1	9	7	9	26
		% within male participants	0.0%	3.8%	34.6%	26.9%	34.6%	100.0%
	Female	Participants count	1	4	4	7	5	21
		% within female participants	4.8%	19.0%	19.0%	33.3%	23.8%	100.0%
Total		Participants of both gender	1	5	13	14	14	47
		% both gender of participants	2.1%	10.6%	27.7%	29.8%	29.8%	100.0%

Distribution of cytological diagnostic categories based on the modified Bethesda grading system was as follows: 13 negative for intraepithelial lesion or malignancy (NILM), 4 atypical squamous cells of undetermined significance (ASCUS), 1 low grade squamous intraepithelial lesion (LSIL), 1 atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASCH), 7 high grade squamous intraepithelial lesion (HSIL) and 21 squamous cell carcinoma (SCC) (table 2). Majority of participants of both gender presented with Squamous Cell Carcinoma (SCC), followed by negative results (NILM) and high grade squamous intraepithelial lesion (HSIL) as shown in table 3 below. Squamous Cell Carcinoma (SCC) was the most predominant cytological finding in most participants aged 40 years and above followed by negative (NILM) results which were fairly distributed among participants of all age groups as shown in table 4. Other significant findings were bacterial and fungal infections which were found on cytological preparations.

Table 2
Cytology results based on modified Bethesda grading system

Cytology Grade		Frequency	Percentage %
	NILM	13	27.7
	ASCUS	4	8.5
	LSIL	1	2.1
	ASCH	1	2.1
	HSIL	7	14.9
	SCC	21	44.7
	Total	47	100.0

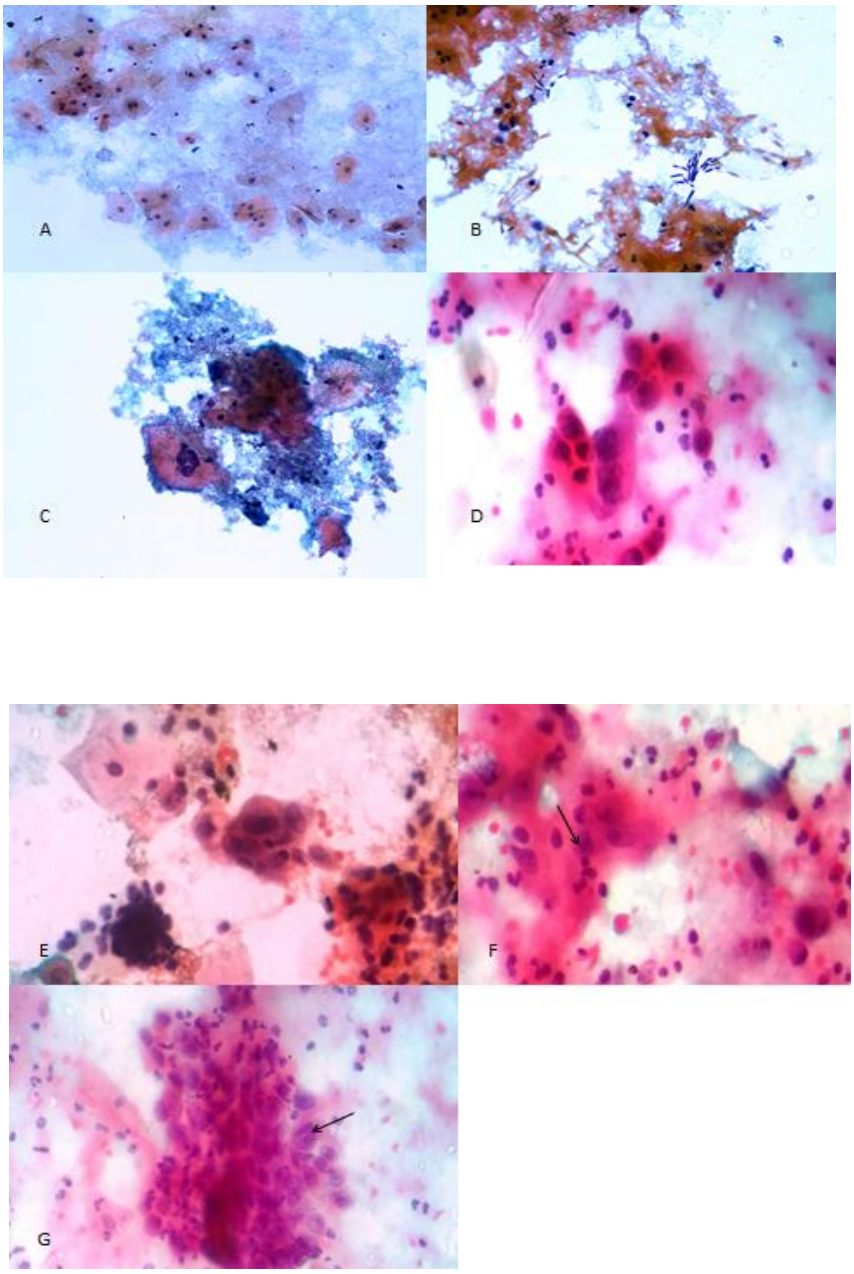
Table 3
Cytology results vs. gender of participants

Gender of participants		Cytology Results						Total
		NILM	ASCUS	LSIL	Asch	HSIL	SCC	
	Male	9	2	0	0	3	12	26
	Female	4	2	1	1	4	9	21
Total		13	4	1	1	7	21	47

Table 4
Cytology results vs. age of participants

Age distribution of participants		Cytology Patterns Results						Total
		NILM	ASCUS	LSIL	ASCH	HSIL	SCC	
	18-28	0	0	1	0	0	0	1
	29-39	3	1	0	0	0	1	5
	40-50	5	1	0	0	2	5	13
	51-61	3	2	0	1	2	6	14
	≥62	2	0	0	0	3	9	14
Total		13	4	1	1	7	21	47

Figure 2
Photomicrographs of oral mucosal cytomorphology patterns



A - Negative for squamous intraepithelial lesion or malignancy (NILM) showing normal intermediate and superficial squamous cells X20
B - Fungal infection consistent with Candida species showing pseudohyphae X40
C - Atypical squamous cells of undetermined significance (ASCUS) showing atypical intermediate squamous cells with multinucleation and bacterial colonies in the background X40
D - High grade squamous intraepithelial lesion micro invasion cannot be ruled out (HSIL)
E - High grade squamous intraepithelial lesion

F and G - Squamous cell carcinoma (SCC) both showing haphazardly arranged loosely cohesive clusters of parabasal sized squamous cells with enlarged hyperchromatic nuclei and prominent nucleoli arrow

Histopathology samples were reported using the squamous intraepithelial neoplasia (SIN) classification system by qualified oral pathologists. Squamous cell carcinoma was the predominant histopathology diagnostic category comprised of 62% of the participants with the rest being no dysplasia histopathological diagnostic category representing 38% as shown in table 5. All the histopathology findings were fairly distributed among all age groups apart from 51 years and above participants in which the majority had squamous cell carcinoma as demonstrated in table 6.

Table 5
Histopathology results

Results		Frequency	Percent
	No dysplasia	18	38.3
	SCC	29	61.7
	Total	47	100.0

Table 6
Cross tabulation of participants' age groups vs. histopathology results

Participants age group		Histopathology Results		Total
		No dysplasia	SCC	
	18-28	1	0	1
	29-39	4	1	5
	40-50	6	7	13
	51-61	4	10	14
	≥62	3	11	14
Total		18	29	47

Histopathology confirmed dysplasia and malignancy in 28 of 30 cytopathologically diagnosed cases (table 7). There were 2 false positive and 1 false negative cases reported in this study as shown on a 2 by 2 table 9 below. The sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of brush cytology using a manual Shandon ® papspin ™ concentration technique and histology as the gold standard was calculated using a 2 by 2 table (table 8) and was 97%, 89%, 93% and 94% respectively as indicated on table 9. The diagnostic agreement (kappa value) was 86.4% with a p value of ≤0.001 with asymptotic standard error of 7.6% (table 9).

Table 7
Cross tabulation of cytology results vs. Histology results

Cytological Results		Histopathology Results		Total
		No dysplasia or malignancy	Invasive SCC	
	NILM	13	0	13
	ASCUS	3	1	4
	LSIL	1	0	1
	ASCH	0	1	1
	HSIL	1	6	7
	SCC	0	21	21
Total		18	29	47

Table: 8
A 2 by 2 table of histopathology vs. cytopathology results

		Histopathology results		
		Positive	Negative	Total
Cytopathology Results summary	Positive	28	2	30
	Negative	1	16	17
	Total	29	18	47

Table 9
Diagnostic performance of brush cytology calculated based on cytology and histology findings n = 47

Sensitivity	97%
Specificity	89%
Positive predictive value	93%
Negative predictive value	94%
The level of agreement Kappa	86.4%
Asymptotic std error and p value	7.6% & ‹0.001

DISCUSSION

A number of established cancer screening programs have shown to significantly reduce patients' morbidity and mortality. Oral mucosal cancer screening is a big challenge in low resource countries. It has been shown that visual examination and vital staining methods have limited value in detecting precancerous and early cancerous lesions. They cannot differentiate dysplastic lesions from benign thus limiting their application for general screening and only being reserved for high risk individuals exposed to known risk factors such as heavy smoking.

In this study we evaluated the performance of brush cytology using a manual Shandon ® papspin ™ concentration method which is a rapid, cheap and non-invasive technique in screening of pre-malignant and malignant oral mucosal lesions utilizing biopsy as the gold standard. This study established that

average age of the participants was 55 years and majority of participants with malignant lesions being 40 years and above. These results were comparable with those found by Mehrotra et al in India who also got a mean age of 55 years and majority of premalignant and malignant lesions were found in participants of age 40 years and above.(12)

The main cytopathological patterns of oral mucosal lesions at Kenyatta National Hospital Dental Clinic (KNH-DC) and the University of Nairobi (UoN) Dental School Hospital are high grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC). Oral brush cytology has a high sensitivity, specificity, positive predictive value and negative predictive value for detecting oral squamous dysplasia and squamous cell carcinoma.

The proportion of dysplasia was 64%, of which low grade dysplasia was 3%, high grade dysplasia was 27% and squamous cell carcinoma was 70%. There were no inadequate or non diagnostic specimens. Infections due to Candida and bacteria were 21%. This is similar to observations by Afrogheh et al in South Africa and Mozaffari et al in Iran who got a 70% dysplastic proportion of which 8% were low grade, 10% high grade dysplasia and 82% squamous cell carcinoma (13,14). The combination of brush cytology and manual liquid based preparation techniques are effective tools for quality cytology and have proved to be good for screening and diagnosis of oral mucosal lesions suspicious for malignancy or malignant.

In this study, the sensitivity, specificity, positive predictive value and negative predictive values for brush cytology technique utilizing Shandon papspin concentration technique was 97%, 89%, 93% and 94% respectively. Afrogheh et al found a sensitivity of 96% which is consistent with our findings (13). However, Mozaffari et al, and Babshet et al found lower sensitivity rates (89% and 84% respectively) (14,15). The observations of this study and Afrogheh et al are attributed to the use of a cervical transepithelial brush (Andwin Scientific – Woodland Hills, CA 91303 USA) for specimen collection. Mozaffari et al and Babshet et al used toothbrushes for specimen collection, which contributed to their disparate findings. Toothbrushes are unsuitable for specimen collection because it does not sample beyond the epithelium and specimens are not easily transferred onto conventional slides. Transepithelial cervical brush (Andwin Scientific – Woodland Hills, CA 91303 USA) should be used in sampling oral mucosal lesions for cytopathology evaluation. The specificity observed in this study was 89% which was consistent with observations of Afrogheh et al and Mozaffari et al who both reported 100% specificity (13,14). We found a higher specificity than Babshet et al and Guneri et al who reported specificity of 83 and 51 % respectively (15,16). Guneri et al did not utilize specimen concentration techniques. Concentration techniques such as manual Shandon ® Papspin ™ liquid based techniques improves the cellularity and should be encouraged in oral exfoliative cytology as its cheap and easy to set up (13,14,17).

The positive predictive value in this study was 93% which was consistent with studies by Afrogheh et al, Mozaffari et al and Babshet et al which were 98%, 100% and 94% respectively (13,15). This study reported higher negative predictive value of 94% than studies by Afrogheh et al, Mozaffari et al and Babshet et al who got 81%, 80% and 63% respectively. Diagnostic agreement kappa value between cytology and histopathology was 86% showing a substantial agreement and was consistent with Mozaffari et al who reported 81% agreement (14).

Although this study is limited by lack of standardized oral cytology reporting systems, this study designed and utilized an evaluation system similar to The Bethesda System for Uterine Cervical Cytology (18).

The assumptions were that the oral mucosa is similar to the uterine ectocervical mucosa (8). The oral mucosa lacks the transformation zone and the etiology of oral squamous cell carcinoma is different from genital squamous cell carcinoma. However, this system is familiar to most pathologists and provides an appropriate and reproducible system.

In conclusion Brush cytology utilizing a manual liquid based Shandon ® papspin ™ technique provides reliable diagnoses for pre-malignant and malignant squamous cell lesions. This has a specificity of 89%, sensitivity of 97%, positive predictive value of 93% and negative predictive value of 94% At Kenyatta National Hospital, the proportion of clinically suspicious lesions showing cytopathological features of dysplasia and invasive malignancy was 64%, of which 30% were dysplastic and 70% had invasive malignancy.

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