

Value of centrifugated liquid-based cytology by Papanicolaou and May-Grünwald in oral epithelial cells

Hussain Gadelkarim Ahmed,¹
Ali Mahmoud Edris,¹
Eneel Ahmed Mohamed,²
Mohammed Omer M. Hussein³

¹Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Khartoum, Sudan;

²Faculty of Dentistry, University of Khartoum, Sudan;

³Sudanese Cancer Society, Khartoum, Sudan

Abstract

For many years, liquid-based cytology (LBC) has been developed for cervical cancer screening and not oral cancer, as it requires automated devices. The aim of this study was to compare the utility of centrifugated CLBC preparation with that of direct preparation in oral lesions, by Papanicolaou (Pap) and May Grünwald-Giemsa's (MGG) methods. A total of 100 consecutive cases of oral lesions were investigated. We compared the results obtained by the CLBC performed by cytocentrifugation with those obtained by direct smear applying Pap and MGG methods. The comparison between CLBC and direct smears was based on the thickening or adequacy of the smear, distribution of cells and staining quality. All smears in CLBC and direct preparation were found adequate. For thickness of the smear, 40% and 42% were excellent, 33% and 30% were good, and 27% and 28% were acceptable by LBC and direct preparation, respectively. For the distribution of cells and scantiness of background elements, 92 (92%) smears of the CLBC have revealed clear, well distributed smears, compared to 70 (70%) of those in direct preparation. For the staining quality with the Pap method, 39% and 69% were excellent staining quality, 25% and 20% were good, and 36% and 11% were acceptable for CLBC and direct preparation, respectively. In MGG method, 9% and 22% were excellent staining quality, 23% and 36% were good and 68% and 43% were acceptable for CLBC and direct preparation respectively. CLBC performed by cytocentrifugation is inexpensive, and reduces inadequate smears and background staining.

Introduction

Oral Exfoliative Cytology (OXC) is a cost effective and perhaps the best procedure for the initial evaluation and diagnosis of oral lesions.¹ It is simple, safe and reliable, especially in population-based screening programs, where repeated samples might be required.² Early detection of a pre-malignant or cancerous oral lesion can improve the survival and the morbidity of patients suffering from these conditions.³

Liquid-based cytology, since its inception in the 1990s, has shown significant advantages over conventional exfoliative cytology. Studies in cervical cytology have shown that the LBC reduces the problems related to sampling and preparation of better smears and reduction in false-negative rates.⁴⁻⁶ Although conventional cytology is useful when diagnosing oral PML (better sensitivity and predictive positive value if compared with the cervical smear test with similar specificity), LBC gives better results, as it is not only enhances both sensitivity and specificity, but also provides material for further investigation (AgNORs, DNA, immunohistochemistry, etc.).^{7,8} LBC using a filtration process and computer assisted thin layer deposition of cells has been developed as a replacement for cytocentrifugation and/or smearing, owing to its improved cell recovery capabilities and better cell preservation. In most published series, LBC allows a good interobserver reproducibility.⁹ However, LBC requires expensive automated devices and materials, which might not be affordable for many cytopathology laboratories in countries with poor resources. Thus, in this study we evaluated the efficiency of the inexpensive CLBC method relying on cytocentrifugation.

Materials and Methods

In this descriptive comparative study, a total of 100 consecutive cases of oral lesions were investigated. Cytological materials were obtained by scraping the surface of the lesion. The obtained materials were used for preparation of two direct smears and the remaining materials were immersed in washing solution for CLBC. One of the direct smears was immediately fixed in 95% ethyl alcohol, while it was wet (for subsequent Pap Stain), and the other was air dried then fixed in methanol (for subsequent MGG stain). The scraped materials for CLBC were flushed out in suspending solution (suspending medium composed of 20 mL of 95% ethanol + 6 mL of glacial acetic acid + 74 mL normal saline (Merck, Darmstadt, Germany); for ten min, and then spun in cytospin for 10 min at 3000 rpm. The

Correspondence: Hussain Gadelkarim Ahmed, University of University of Khartoum, 102, Faculty of Medical Laboratory Sciences, Khartoum, Sudan
E-mail: juhina2005@gmail.com

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formed supernatant was poured off and replaced by acid alcohol for 30 min. Then the supernatant was discarded leaving only a few drops which were shook vigorously with acid alcohol. Thereafter, a drop of coating medium (glycerin/albumin) was added. Then two smears (wet fixed and air dried) were made from each specimen on moist, clean glass slides. The slide was tilted and with a Pasteur pipette, pellet was taken and replaced at the upper end of the slides, left to drain, and then left to dry overnight.

Direct preparation and CLBC smears were stained using staining methods (Pap and MGG). For the smears which were stained using the Papanicolaou method, ethyl alcohol fixed smears were hydrated in descending concentrations of 95% alcohol through 70% alcohol to distilled water for 2 min in each stage. Then smears were treated with Harris' Haematoxylin for 5 min, to stain the nuclei, rinsed in distilled water and differentiated in 0.5% aqueous hydrochloric acid for a few seconds to remove the excess stain. They were then immediately rinsed in distilled water to stop the action of discoloration. Then

the smears were blued in alkaline water for a few seconds and dehydrated in ascending alcoholic concentrations from 70% through two changes of 95% alcohol for 2 min for each change. The smears were next treated with eosin Azure 50 for 4 min. For cytoplasmic staining they were treated with Papanicolaou Orange G6 for 2 min, rinsed in 95% alcohol and then the smears were dehydrated in absolute alcohol. The smears were then cleared in Xylene and mounted in DPX (Distrene polystyrene Xylene) mount. All reagents used were from Thermo Electron Corporation, UK.

For the smears which were stained using the MGG method, the air dried methanol fixed smears were transferred to a staining jar containing May Giemsa stain freshly diluted with an equal volume of buffered water for 15 min, then transferred without washing to a jar containing Giemsa stain freshly diluted with nine volume of buffered water for 10 min. The smears were then washed rapidly in three changes of water and examined.

Assessment of cytological smears for staining quality

The smears were assessed and evaluated by an experienced cytotechnologist. For comparative analysis of both techniques, parameters such as thickness, cellular distribution, leukocytes and red blood cells were evaluated, adopting criteria reported elsewhere.^{10,11} Also, given that a good staining method must show the shapes and sizes of the cell, provide crisp delineation of nuclear chromatin, and demonstrate the cytoplasm, each slide was given a mark out of ten and graded as follows: (i) 10-8 excellent; (ii) 7-5 good; (iii) 5 acceptable. All parameters were compared to standard parameters illustrated elsewhere,¹² and the degrees were given.

We compared the results obtained by the centrifugated liquid-based cytology (CLBC) diagnoses performed by cytocentrifugation with those obtained by direct smear applying Pap and MGG methods. The comparison between CLBC and direct smear was based on the thickening or adequacy of the smear, distribution of cells and staining quality.

Results

As the comparison between CLBC and direct smears was based on the thickening or adequacy of the smear, distribution of cells, and scantiness of background elements and staining quality, all smears in CLBC and direct preparation were found adequate, though few 5 (5%) of direct preparation showed a reduced amount of cells. With regard to the thickening

of the smear, 40% and 42% were excellent thickness, 33% and 30 were good, and 27% and 28% were acceptable by CLBC and direct preparation, respectively. For the distribution of cells and scantiness of background elements, 92 (92%) smears of the CLBC have revealed clear, well distributed smears, compared to 70 (70%) of those in direct. However, 8 (8%) and 30 (30%) of the CLBC and direct smears, respectively, have shown a disorganized pattern.

When comparing the staining quality between the CLBC and direct smears, with the Pap method 39% and 69% were excellent staining quality, 25% and 20% were good, and 36% and 11% were acceptable for direct preparation and CLBC, respectively. With the MGG method, 9% and 22% were excellent staining quality, 23% and 36% were good, and 68% and 43% were acceptable for direct preparation and CLBC, respectively. When comparing the staining quality between Pap and MGG in direct preparation, 39% and 9% were excellent staining quality, 25% and 23% were good, and 36% and 68% were acceptable by Pap and MGG, respectively. When comparing the staining quality between Pap and MGG in CLBC, 69% and 22% were excellent staining quality, 20% and 36% were good, and 11% and 43% were acceptable by Pap and MGG in this order, as shown in Table 1.

Discussion

Oral cancer (OC) mortality is very high in the Sudan, particularly among men due to the habit of Toombak use [Tobacco Specific Nitrose amine (TSN) rich tobacco].¹³ Toombak dippers develop a clinically and histologically characteristic lesion at the site of dipping. The risk for cancer of the oral cavity among Toombak users is high (RR 7.3-73.0-fold)¹⁴ Therefore, there is an urgent need for implementation of simple and cost-effective methods to screen the population at risk.

Oral exfoliative cytology is a non-aggressive procedure that is well accepted by the patient, and is, therefore, a suitable choice for the early diagnosis of oral cancer, including epithelial atypia and squamous cell carcinoma.¹⁵ In recent years, LBC has acquired a wide range of acceptance in non-cervical cytology specimens,¹⁶ including oral cytology.¹⁷ This method is convenient in interpreting the results since it yields optimal cellularity for evaluation, and studies have shown similar or even better diagnostic accuracy as compared to the direct smear method.^{10,18} In this study, 92 (92%) of the smears of the CLBC have revealed clear, well distributed smears, compared to 70 (70%) of those in direct prepara-

Table 1. Showing the comparison between direct preparation and LBC using Pap and MGG methods.

	39 (39%)	9 (9%)
Direct		
Excellent	39 (39%)	9 (9%)
Good	25 (25%)	23 (23%)
Fair	36 (36%)	68 (68%)
CLBC		
Excellent	69 (69%)	22 (22%)
Good	20 (20%)	36 (36%)
Fair	11 (11%)	43 (43%)

tion. CLBC showed thin uniform distribution of cells, in addition to clear background due to reduction in both cell overlapping and the presence of artifacts. The cells also appeared well preserved in their morphology and this might be due to obtaining sufficient fixation and the release of artifacts by washing. In regard to the thickening of the smear, both techniques achieved similar appearance, and we think that thickening of the smear depends to some extent on the skilful preparation of the smear. However, some studies reported that the scantiness of background staining obtained in LBC enhances sensitivity and quality.¹⁹ Not surprisingly studies of the accuracy of liquid-based monolayer cytology report sensitivity of 61% to 66% and specificity of 82-91%.^{20,21} Furthermore, comparable results between LBC and direct preparation have been reported.²²

When comparing the staining quality (using Pap and MGG stains) between the LBC and direct smears, CLBC preparation has shown superior staining quality compared to that of direct preparation. Cellular details in CLBC were more clearly seen than in direct preparation, and such findings were previously reported applying automated LBC.²³ However, some studies have found no significant difference between LBC and conventional cytology.¹¹ However, many studies have reported the reliability of Pap stain compared to other cytological stains by the means of differentiating and identifying cellular details.^{24,25}

When comparing the staining quality between Pap and MGG in CLBC and in direct preparation, Pap stain revealed better staining quality. A study by James *et al.*²⁶ found an agreement between Pap and MGG stain analyses with regard to specimen adequacy.

Both, the liquid-based preparation and conventional smear are diagnostically reliable; the liquid-based method showed an overall improvement on sample preservation, specimen adequacy, visualization of cell morphology and reproducibility.

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