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Research Article

Effectiveness of Rapid, Economic, Acetic Acid, Papanicolaou Stain (REAP) Over Conventional Papanicolaou Stain in Oral Exfoliative Cytology

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Abstract

The study of cells that exfoliate from body surfaces is known as exfoliative cytology. These exfoliated cells, as well as cells scraped off with certain instruments, can be quantitatively or qualitatively analyzed. Papanicolaou (PAP) staining is the routinely used diagnostic test in case of suspicious oral smears. The Rapid, Economic Acetic acid Papanicolaou (REAP) staining technique was found to be superior to the conventional PAP stain in cervical cytology.

Aim: To assess the effectiveness of REAP technique as a better chairside diagnostic procedure than the conventional PAP technique in oral exfoliative cytology.

Materials and Methods: A total of 100 smears were collected from 50 patients clinically diagnosed with potentially malignant disorders indicated for oral exfoliative cytology. Both sets of smears were stained, one using the conventional PAP method and the other using the REAP method. The two sets of smears were observed by two trained pathologists and evaluated for staining quality. The time for staining was also assessed. Over a 15-month observation period, the color stability of the smears stained by PAP and REAP were compared.

Statistical Analysis: Chi-square test was used to assess the significance of the difference between proportions of optimal staining between the two methods. Quantitative parameters between techniques were compared using the independent sample t-test.

Results: The staining quality of REAP smears was better than conventional PAP and was statistically significant ($p < 0.001$). The staining time of REAP is completed in 7 minutes whereas the PAP technique took 30 minutes. Both REAP and PAP smears retained their colour stability for 15 months.

Conclusion: REAP technique is a better chair-side diagnostic procedure than the conventional PAP technique in oral exfoliative cytology as it produces better staining quality and involves minimal time. The colour preservation of REAP stained smears is also excellent.

Keywords: Oral Exfoliative Cytology; REAP; Conventional PAP

Introduction

Normal epithelium exfoliates its superficial cells as a result of physiological turnover. When the epithelium becomes seat of any pathological condition, the cells of deeper layers may lose their cohesiveness and may shed along with the superficial cells. These exfoliated cells as well as cells which are scrapped off by means of specific instruments, can be studied quantitatively or qualitatively [1].

Papanicolaou (PAP) staining, which produces a polychromic, transparent staining reaction with distinct nuclear/cytological features, is a frequently used cytological method to rule out oral cancer. It was first described by Papanicolaou and commonly used as a screening tool in spite of being tedious and using a significant amount of expensive alcohol. Various articles describe modifications of Papanicolaou staining, such as Ultra-Fast1 and Rapid Pap. These are rapid techniques and staining is achieved in 90 seconds,

but in these, substantial volumes of ethanol are used [2]. The REAP technique was found by Dighe SB and was shown to be superior to the conventional PAP stain. The REAP stain produces better staining quality with precise cellular details. It is economical since acetic acid is used instead of ethanol and rapid technique with good colour stability. Hence, REAP was proved to be cost-effective and quicker procedure [3].

The present study was done to assess the effectiveness of REAP technique as a better chair-side diagnostic procedure than the conventional PAP technique in oral exfoliative cytology.

Materials and Methods

The study was conducted at Govt. Dental College Thiruvananthapuram, and was approved by institutional ethics committee. A total of 100 smears were collected from 50 patients clinically diagnosed with potentially malignant disorders indicated for oral exfoliative cytology. One set of smears were stained with conventional PAP technique and the other set with the REAP technique. The two sets of smears were observed by two trained pathologists and assessed for staining quality. The time for staining was also assessed.

Method of smear preparation

Informed written consent was taken from the patients, he/she was asked to rinse the mouth with water before taking the smear. The oral mucosa was gently scraped with cytobrush and the material submersed in 5ml of saline solution (0.9% NaCl). The saline solution containing the exfoliated cells was subjected to centrifugation for 10min at 15000 rpm. Following centrifugation, the supernatant was discarded while the pellet of cells was spread on two clean glass slides and fixed in 3:1 methanol/acetic acid for 15min. Both sets of smears were stained, one using the conventional PAP method and the other using the REAP method.

Conventional PAP technique

- Transfer slides to ether fixative without drying and bring down to descending grades of alcohol and distilled water.
- Transfer slides to Harris hematoxylin and stain for 4 minutes
- Wash in distilled water
- Immerse slides in 0.25% HCl about six times
- Wash in tap water for 6 minutes
- Wash in distilled water and put through ascending grades of alcohol
- Transfer slides to Orange G-6 and stain for 2 minutes
- Bring down the slides to two changes of 95% alcohol
- Transfer slides to EA36 and stain for 2 minutes
- Wash the slides in three changes of 95% alcohol. Transfer the slides in 100% alcohol and bring down the slides equal parts absolute alcohol and xylol. Immerse the slides in xylol to clear and mount the slides.

Alterations in REAP technique in contrast with PAP

- Ethanol is replaced by 1% acetic acid.
- Haematoxylin is heated to 60oC before staining.
- Methanol is used instead of absolute alcohol.
- Blotting will be done after each step.
-

Evaluation of staining

The REAP technique was standardized for oral smears. The cytoplasmic and nuclear staining of both techniques were compared based on the following criteria.

- Cell /cytoplasmic borders (distinct/indistinct),
- Cytoplasmic staining (satisfactory/unsatisfactory),
- Nuclear borders (distinct/indistinct) and
- Chromatin staining (distinct/hazy).

If the above criteria were satisfied, then the staining quality would be optimal and if the above criteria were not satisfied, then the staining quality would be suboptimal.

All of the stained smears were read by two trained pathologists who were blinded about the staining technique. The colour stability of the smears stained by both techniques was compared for 15 months. The staining time for each respective smear was compared.

Results

A total of 100 smears stained by conventional PAP and REAP stain were assessed. The optimal and suboptimal staining for each technique was assessed. Table 1 and Figure 1 shows the comparison between staining quality of REAP and conventional PAP technique. In REAP technique staining quality was optimal in 92% of cases and suboptimal in 8% of cases whereas in conventional PAP technique staining quality was optimal in 90% of cases and suboptimal in 10% of cases. According to the statistical analyses the staining quality of REAP smears was better than conventional PAP stain and was statistically significant ($p < 0.001$).

Table 2 compares the nuclear and cytoplasmic staining quality of both REAP and conventional PAP technique. The cell/cytoplasmic borders were distinct in 96% of REAP stained smears and indistinct in 4% cases. In conventional PAP smears, the cell/cytoplasmic borders were distinct in 90% of cases and indistinct in 10% of cases (Figure 2,7). The cytoplasmic staining was satisfactory in 94% of REAP stained smears and unsatisfactory in 6% cases (Figure 6). In conventional PAP smears, the cytoplasmic staining was satisfactory in 92% of cases and unsatisfactory in 8% of cases (Figure 3). The nuclear borders were distinct in 92% of REAP stained smears and indistinct in 8% cases. In conventional PAP smears, the nuclear borders were distinct in 94% of cases and indistinct in 6% of cases (Figure 4). The chromatin staining was distinct in 94% of REAP stained smears and hazy in 6% cases. In conventional PAP smears, the chromatin staining was distinct in 90% of cases and hazy in 10% of cases (Figure 5). According to the value of measure of agreement kappa for each parameter of staining quality, it is evident that REAP is an equally effective staining technique as conventional PAP.

The staining time for both techniques was compared and it was 7 minutes for REAP while 30 minutes with PAP stain (Table 3).

The colour stability of each smears were compared over one year and it remained well preserved (Table 4).

Procedure	Optimal Staining (%)	Suboptimal Staining (%)
REAP	92	8
PAP	90	10

Table 1: Comparison of staining quality of REAP and conventional PAP staining technique.

Parameter		PAP (%)	REAP (%)	Measure of agreement Kappa	p
Cell/Cytoplasmic borders	Distinct	90	96	0.545	<0.001
	Indistinct	10	4		
Cytoplasmic staining	Satisfactory	92	94	0.847	<0.001
	Unsatisfactory	8	6		
Nuclear borders	Distinct	94	92	0.847	<0.001
	Indistinct	6	8		
Chromatin staining	Distinct	90	94	0.730	<0.001
	Hazy	10	6		

Table 2: Comparison of nuclear and cytoplasmic staining quality of REAP and conventional PAP staining technique.

Procedure	Turn around time
REAP	7 minutes
PAP	30 minutes

Table 3: Comparison of turnaround time of REAP and conventional PAP staining technique.

Procedure	Smear preservation (1 year)
REAP	Excellent
PAP	Excellent

Table 4: Comparison of smear preservation of REAP and conventional PAP staining technique.

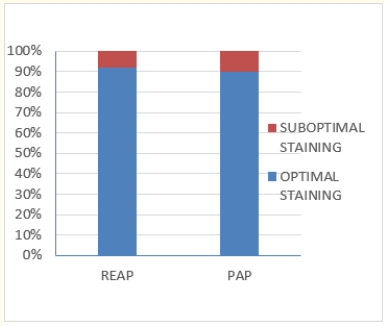

Figure 1: Comparison of staining quality of REAP and conventional PAP staining technique.

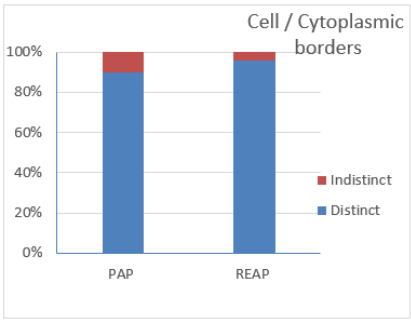

Figure 2: Comparison of cell/cytoplasmic borders of REAP and conventional PAP stained smears.

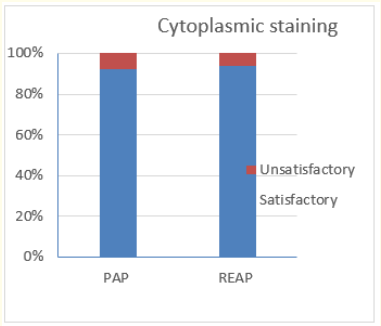

Figure 3: Comparison of cytoplasmic staining of REAP and conventional PAP stained smears.

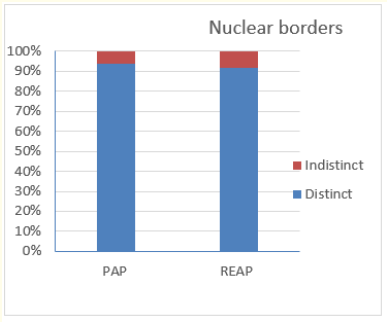

Figure 4: Comparison of nuclear borders of REAP and conventional PAP stained smears.

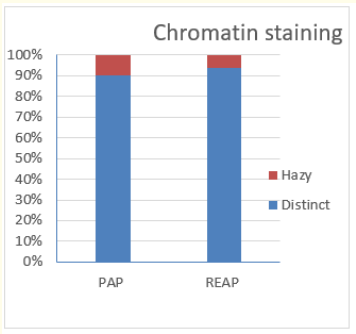

Figure 5: Comparison of chromatin staining of REAP and conventional PAP stained smears.

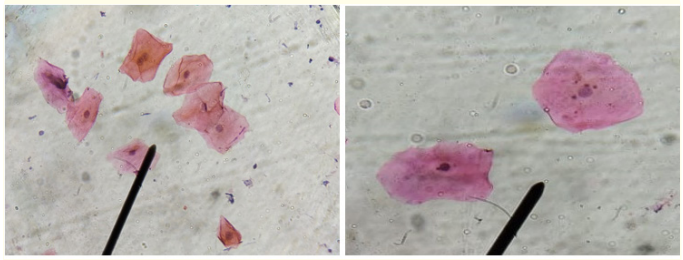

Figure 6: REAP stained smear showing optimal differentiation and transparency of the cytoplasm with crisp and clear nuclear details and chromatin pattern.

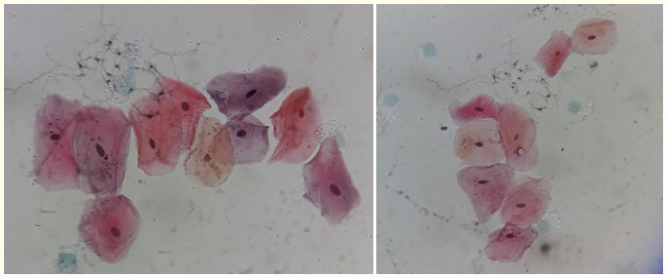

Figure 7: PAP smear showing optimal differentiation and transparency of the cytoplasm with crisp and clear nuclear details and chromatin pattern.

Discussion

The universal stain for oral cytological screening for precancer and cancer of oral cavity is Papanicolaou stain [4]. Pap stain was first introduced by Papanicoloau to assess the variation in cellular maturity in cervical smears [5]. The initial Pap stain was later modified by him in 1954 and 1960 [6]. It yields a multichromatic, transparent staining reaction with distinct cellular features [7]. The conventional Papanicolaou staining method is tedious and technique sensitive. As a result of above reasons Papanicolaou staining method has been modified in several ways [8]. The various methods tried are, Ultra - fast staining, Rapid PAP, Quick PAP staining. These alternative methods are rapid, but use expensive ethyl alcohol [9]. The REAP stain is rapid and provides better cellular details but it utilizes a significant amount of costly ethanol. In REAP, 1% acetic acid is used instead of ethanol [7]. These advantages had made this technique a better alternative for conventional PAP [10,11].

The staining was evaluated by considering cell borders, cytoplasmic staining, nuclear borders and chromatin staining, which was graded into optimal and suboptimal. According to the present study, in REAP technique, staining quality was optimal in 92% of cases and suboptimal in 8% of cases whereas in conventional PAP technique staining quality was optimal in 90% of cases and suboptimal in 10% of cases. According to the statistical analyses the staining quality of REAP smears is better than conventional PAP stain and is statistically significant ($p < 0.001$).

In REAP, 1% acetic acid was used instead of ethanol. It acts as a nuclear fixative and also produces crisp nuclear features. Thus, the staining quality was superior in the REAP stained smears and was statistically significant in contrast with conventional PAP.

Both OG6 and EA36 are ethanol based cytoplasmic stains. In PAP, when the smears were dehydrated in ethanol which results in the diffusion of some of the stain into ethanol. Consequently, the cytoplasmic staining intensity declines. But in REAP, ethyl acetate is formed as result of chemical reaction between acetic acid and ethanol. It combines with OG6 and EA36 and is collected in the cells, maintaining the stain intensity. Hence, the cytoplasmic staining is equivalent to PAP.

The staining time of PAP and REAP for each respective smear was compared. The staining time for both techniques was compared and it was 7 minutes for REAP while 30 minutes with PAP stain. The colour stability of each smears were compared over one year and it remained well preserved.

Asthana., *et al*. conducted a study to compare the staining of oral smears by REAP technique and PAP technique. According to this study, the cytoplasmic staining in REAP was optimal in 84% smears and in 16% smears it was suboptimal. The nuclear staining was compared between REAP and PAP smears which was optimal in 92% REAP smears. It was suboptimal in 8% of cases. The staining time was 3-4 minutes for REAP as compared to 18-20 minutes with PAP stain. The staining quality of all the REAP smears remained well preserved for more than 1 year. They concluded that REAP staining is superior to PAP staining as it provides smears with better staining quality and time saving procedure.

Conclusion

The present study shows that REAP technique is a better chair side diagnostic procedure than conventional PAP technique in oral exfoliative cytology. The REAP stain offers greater staining quality, quicker procedure, excellent colour stability and economical in contrast with conventional PAP stain.

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