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Rapid economic acetic acid papanicoloau (REAP) stain: Is it truly rapid and economical enough to replace conventional papanicolaou stain?

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Abstract:
Background*: Routine Papanicolaou (PAP) staining is a commonly employed cytological procedure, but procurement of stain's prime constituent ethanol is costly and method is time consuming too. On other hand rapid economic acetic acid papanicolaou (REAP) technique yields excellent nuclear and cytoplasmic staining, is cheaper and quicker procedure compared to routine PAP.*
Aims*: Our aim was to assess the superiority of REAP over routine PAP and assessing its applicability.*
Methods and Material*: The prospective cross sectional study was carried out on 70 patients in gynecology opd during period of 1year. Two smears from each patient were collected and stained by routine PAP and REAP stain separately.*
Results*: Majority of patients were of 31-35 yrs age group. 67 cases were observed to have satisfactory cytoplasmic and nuclear staining by REAP in comparison to 61 and 60 cases respectively by routine PAP. REAP technique was found to be statistically superior in cytoplasmic and nuclear staining with p value of 0.02 and 0.03 respectively(OpenEpi,operating system). Cost of REAP was also reduced as absolute alcohol was replaced by cheaper 1% acetic acid. Time taken was also reduced to (10 ± 3mins) due to simplicity and uniformity of the procedure. Preservation of the slides was also good till 6 months as ethyl acetate preserves the cytoplasmic staining and acetic acid acts as nuclear stain fixative.*
Conclusions*: REAP technique stands true to its name and is undeniably better than routine PAP. It provides crisp nuclear and cytoplasmic staining, is time saving and cost-effective with long term color preservation. Thus can be utilized for screening in low resource setups.*
Key-words*: Acetic acid, Cervical cancer, Ethyl alcohol, PAP stain*

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I. Introduction:

Cervical cancer is a major cause of death worldwide, with approximately 4,90,000 women diagnosed annually with invasive cervical cancer and accounts for over 2,30,000 deaths annually. The majority of cases (80%) occur in developing countries where it is the second most common cancer after breast cancer.[1] It is preceded almost without exception by precancerous lesions that develop over several years.[2] Cervical cancer is preventable and screening methods exist to detect it at a precancerous state. Cytology-based PAP smear is one such reliable tool.[3] Epidemiological data show that annual screening reduces the mortality by 70% and the probability of developing invasive carcinoma is reduced by over 95%.[4] Standard PAP smear has been the most successful cancer screening test in history since its introduction in the 1950s, although it uses a substantial quantity of alcohol which hinders its use as a mass screening tool in low-resource settings. Various modifications have been done in order to reduce time, alcohol use and improve the staining quality.[5,6,7]

Over years cytopathologist have felt that due to increasing burden of cancer screening programme, PAP stain is proving to be less cost effective, cumbersome and time consuming. Hence, there is search for a less expensive and rapid stain, but at the same time it does not compromises the quality.[8] REAP is a modification of standard PAP which is defined as Rapid Economic Acetic acid Papanicolaou stain. As the name implies, the technique is rapid, economical, acetic acid is used as a dehydrant and colour preservation. These qualities had made this technique superior than that of standard PAP.[9,10]

The present study aims to ascertain superiority and advantages of REAP over conventional PAP stain along with its applicability in cyto-morphological study of cervical scrape smear.

Subjects and Methods: It is a prospective cross sectional study which was carried out on 70 female patients in out patient department of Obstetrics and Gynaecology, during period of 1yr (March 2015 to March

2016). Cervical scrape smears from sexually active adult female patients (18 - 49 yrs age) who did not undergo total hysterectomy and coming for cervical screening were taken into account. Two smears from each patient were collected and stained by standard PAP and REAP method of stain separately. Thus total 140 slides (70 × 2) were stained.

REAP stain was introduced by S.B Dighe in 2005.[11] The principle is to clearly distinguish between basophilic and acidophilic cell components and obtain a detailed chromatin pattern. Even though UFP and Rapid PAP takes less time for staining, it requires large quantity of alcohol and hence it is expensive. To overcome this, REAP was prepared which takes approximatety 7 mins and 1% acetic acid replaces 95% alcohol. Hence, acetic acid acts as mild dehydrating agent, nuclear fixative, increases staining intensity of nucleus, preserve stain colour, does rapid staining and is cheap as well as easily available.

Technique: Method for REAP stain :[7]

1. Fixation
   - 15-20 mins in Absolute alcohol.
2. Nuclear staining
   - 1%Acetic acid, 10 dips.
   - Harris's Haematoxylin, preheated to 60° C, 10 dips.
   - Tap water, 10dips.
   - 1%Acetic acid, 10 dips.
3. Cytoplasmic staining
   - OG-6, 10dips.
   - 1% Acetic acid, 10 dips.
   - EA-50, 10 dips.
   - 1%Acetic acid, 10 dips.
   - Methanol /Absolute alcohol, 10 dips.
4. Clearing and mounting
   - Xylene 10 dips.
   - Blotting done after each step.
   - Mount by D.P.X.

II. Results:

All the cases stained with conventional and REAP staining technique were evaluated by two pathologists at different time and the final diagnosis and quality of staining was compared with that rendered by the reporting consultant. Patients ranging from 21-50 yrs age group came for PAP smear screening as enumerated in table 1. However maximum number of patients were from 31-35 yrs (42.86%) age group.

Table -01: Age wise distribution

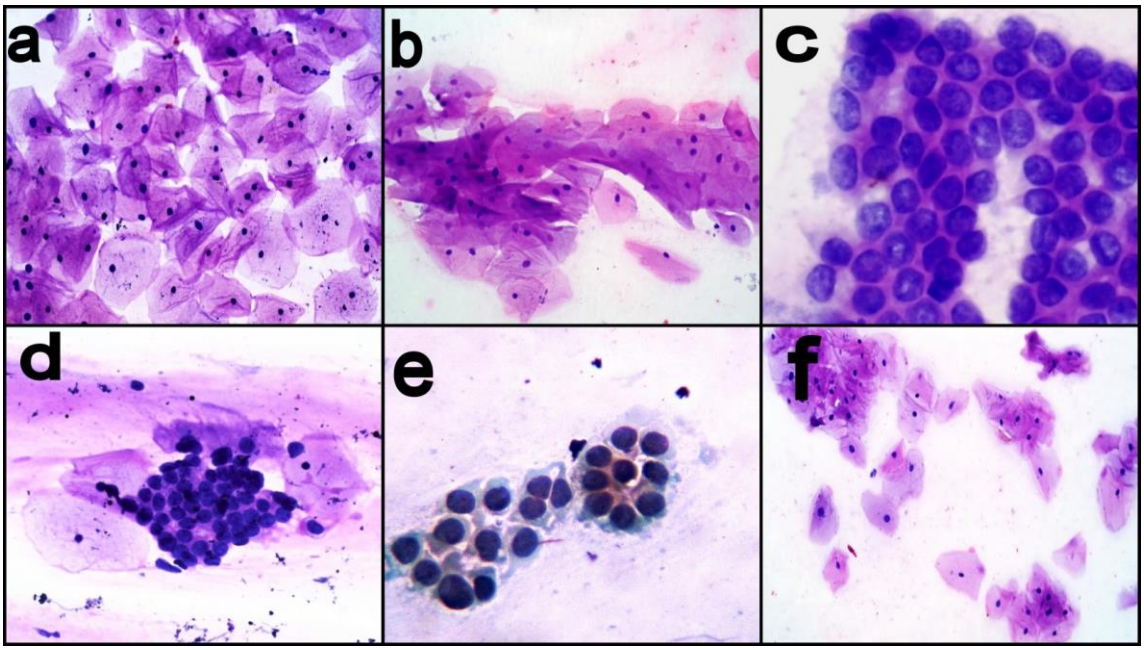
Age group	No. of patients	%
21-25 yrs	5	7.14
26-30 yrs	10	14.29
31-35 yrs	30	42.86
36-40 yrs	12	17.14
41-45 yrs	9	12.86
46-50	4	5.71
Total	**70**	**100.00**

Out of 70 cases stained with PAP and REAP, cytoplasmic stain was evaluated on the basis of whether it was satisfactory or unsatisfactory as described in table 2. Sixty one (87.1%) cases stained with PAP and 67 (95.7%) cases stained by REAP showed satisfactory cytoplasmic stain as shown in figure 1(a). There was statistical significance (p value- 0.03), (OpenEpi,operating system) between two staining techniques. Cytoplasmic border was evaluated on the basis of whether it was distinct or indistinct. 67 (95.7%) cases stained with both REAP and PAP showed distinct cytoplasmic border while 03 (4.2%) cases stained with both stains showed indistinct cytoplasmic border. There was no statistical difference in assessing cytoplasmic border (p value - 0.99) between the two staining techniques. Nuclear chromatin was evaluated on the basis of whether it was distinct or hazy. 60 (85.7%) cases stained with PAP and 67 (95.7%) cases stained with REAP showed distinct nuclear chromatin as shown in figure 1 (c) and (e). Thus there was statistical significance between the two (p value-0.02). Nuclear border was evaluated on the basis of whether it was distinct or indistinct. 66 (94.2%) cases stained with PAP and 67(95.7%) cases stained with REAP showed distinct nuclear border. Thus both the staining techniques were nearly similar in distinction of nuclear borders & there was no statistical difference (p value - 0.36) between the two techniques.

Table 02: Comparison of staining quality by both techniques.

Parameters	PAP		REAP	
	No	%	No	%
1. Cell/cytoplasmic border				
Distinct	67	95.7	67	95.7
Indistinct	03	4.2	03	4.2
2. Cytoplasmic staining				
Satisfactory	61	87.1	67	95.7
Unsatisfactory	09	12.8	03	4.2
3. Nuclear border				
Distinct	66	94.2	67	95.7
Indistinct	4	5.7	03	4.2
4. Nuclear chromatin				
Distinct	60	85.7	67	95.7
hazy	10	14.2	03	4.2

It was also observed that cost of REAP was ¼ of PAP due to replacement by cheaper reagent acetic acid. Routine PAP required 2.5 L of absolute alcohol to stain 100 slides. 1L absolute alcohol costs 800 Rs, thus total cost of alcohol comes out to be (2.5× 800= 2000 Rs). Per slide cost by PAP stain was 20 Rs. REAP required 600ml of absolute alcohol to stain 100 slides. Total cost of alcohol here comes out to be (0.6× 800= 480 Rs). Thus per slide cost by REAP stain was 4.8≈ 5Rs. Preservation was good till 6 months by REAP while slides stained by routine PAP faded after 3months of the staining procedures. Staining time by REAP ranged between 5-7 minutes in comparison to 20-25 minutes by conventional PAP.



Legends:
Figure 1: A. Superficial squamous cells with satisfactory eosinophilic cytoplasmic stain (REAP, 200X)
B. Conventional PAP stained smear of A (PAP, 200X)
C.Endocervical cells showing distinct nuclear stain (REAP, 400X)
D. Conventional PAP stained smear of C (PAP, 400X)
E. Squamous metaplastic cells with distinct nuclear amd satisfactory cytoplasmic stain (REAP, 400X)
F. Clear background in REAP stained smear (200X)
Other additional findings noted in our study were that the background of smears stained by REAP was extremely clear, without any debris as shown in figure 1(f). There was no difference in the staining reaction of non-epithelial cells, such as white blood cells in either staining technique. Thick smears took little longer time than usual to stain (increase 5 dips in each step). The diagnosis of 67 cases after staining with both methods were rendered same , however in three cases infections were missed by REAP staining (two bacterial vaginosis and one trichomoniasis).

III. Discussion:

After the introduction of conventional stain by George Papanicolaou, it underwent various modifications. REAP technique is modification of standard PAP technique which is defined as Rapid Economic Acetic acid Papanicolaou stain. As the name implies, the technique is fast, cost effective and acetic acid is used as a dehydrant and colour preservative.[3]

In present study largest subgroup belongs to 31-35 yrs age, this is possibily because patients are of reproductive age group and chances of sexually transmitted diseases, infections and probability of occurrence of any premalignant lesions are more common in this age group.

REAP was found to be superior to conventional PAP in cytoplasmic staining and similar results were achieved by Biswas et al and Gachie et al. [7,12] Superiority is due to use of 1% acetic acid which acts as dehydrating agent, thus a chemical reaction occur between acetic acid and ethanol (from OG6 and EA36). This reaction leads to formation of ethyl acetate and water. Since most of the water is removed from the cell during the reaction, the ester complexes with the cytoplasmic stains and is deposited in the cells, subsequently preserving staining intensity.

In present study, REAP was found superior to conventional PAP in staining of nuclear chromatin. Similar results were observed in previous studies by Biswas et al and Gachie et al. [7,12] It is because in REAP, pre heated (60^0 C) harris haematoxylin was used, acid differentiation step was discarded and 1% acetic acid was used as the dehydrating agent in place of ethanol. 1% acetic acid also acts as a nuclear fixative which intensifies the staining intensity therefore the nuclear staining in case of REAP was better than PAP.

REAP was found almost similar to conventional PAP in staining of cytoplasmic and nuclear borders. 67 cases showed distinct cytoplasmic borders and nuclear borders respectively by REAP while 67 cases showed distinct cytoplasmic border and 66 cases showed distinct nuclear border by conventional PAP stain. This miniscule difference in interpretation which was statistically insignificant may be due to inadequate sample size and overlapping/ thick smear preparations. Similar observations were also made by Deshpande et al. [8]

In present study cost of REAP was about ¼ of conventional PAP, i.e 5 Rs per slide. Similar results were observed by almost all previous studies. [3,7,8,11] This reduction is due to the fact that amount of alcohol used was considerably reduced and in our country purchase of absolute alcohol in bulk requires license. Obtaining license and its annual renewal is a hazardous task.

Preservation of smears was excellent till 6 months in comparison to conventional PAP. It is because ethyl acetate preserves the cytoplasmic staining, acetic acid also acts as a nuclear stain fixative, preserving the nuclear staining. [7,8,11]

Time taken for REAP was also considerably reduced. Conventional PAP had taken 25± 5mins while REAP required 10 ± 3mins for completion. The simplicity of the procedure (uniform 10 dips at each step) reduced the risk of errors while staining because there is no variation in time or dips from one staining container to the other unlike in the standard PAP where each container has different timings during staining. [3,5,7,8,11]

So REAP stain is genuine to its name and is rapid and economical tool which does not alter cell morphology thus can be used in low resource rural settings where screening load is disproportionate. Easy procurement of acetic acid which is the prime constituent in REAP, simplicity and rapidity of procedure makes it suitable alternative for mass screenings of cervical smears in developing country like India.

To conclude REAP provided satisfactory cytoplasmic and nuclear staining than conventional PAP. The use of absolute alcohol is minimized as it is used only during fixation and for dehydration before mounting, thus making it affordable . The stain preservation is also good till 6 months and time for staining was also reduced to 10±3mins. It may be considered the suitable alternative to routine PAP for cervical cancer screening programme in our country with high prevalence of the disease. Hence REAP in comparison to routine PAP, provides a suitable, excellent & rapid alternative for cytological screening with minimum cost.

References

[1]. Van Hamont D, Bekkers RL, Massuger LF, Melchers, WJ. (2008). Detection, management, and follow-up of pre-malignant cervical lesions and the role for human papillomavirus. Reviews in Medical Virology, 18(2), 117–132.
[2]. Cochand PB, Legales C, De cremoux P, Molinie V, Sastre G X, Vacher L-MC. "Cost-effectiveness of Monolayers and Human Papillomavirus Testing Compared to That of Conventional Papanicolaou Smears for Cervical Cancer Screening: Protocol of the Study of the French Society of Clinical Cytology." Diagnostic Cytopathology, 24 (6), 412-20..
[3]. Asthana S, Satyanarayana L. Prevention and control of uterine cervical cancer, current strategies.Indian J. Community Med. 2007; 32: 233-234.
[4]. Nguyen HN, Staffan, Nordqvist RB. The Bethesda system and evaluation of abnormal PAP smears oncology associates, Sheridan Health Care Corporation, Hollywood, Florida Gynecologic Seminars in Surgical Oncology. 1999; 16: 217–221.
[5]. Gupta S, Chachra KL, Bhadola P, Sodhani P . Modified Papanicolaou staining protocol with minimum alcohol use: A cost-cutting measure for resource limited settings.Cytopathology, 21 (4), 229-233.
[6]. Gill GW. Enviro-PAP: an environmentally friendly,economical, and effective PAP stain. Lab. Med.Indianapolis. 2006;37: 105–108.
[7]. Biswas RR, Paral CC, Dey R, Biswas SC. Rapid economic, Acetic Acid, Papanicolaou Stain (REAP) – Is it suitable alternative to standard PAP stain? J. Med.Sci. 2008; 1(2):99-103.

[8]. Deshpande AK, Bayya P, Veeragandham S. "Comparative Study of Papanicolaou Stain [PAP] with Rapid Economic Acetic Acid Papanicolaou Stain (REAP) in Cervical Cytology". Journal of Evolution of Medical and Dental Sciences 2015; 4(41): 7089-7095.
[9]. Bibbo M. Comprehensive Cytopathology. Philadelphia: WB Saunders.1991;(2):881-906.
[10]. Koss LG. Diagnostic cytology and its histopathologic basis. New York:J B Lippincott.1992;5:1452-1509.
[11]. Dighe SB, Dulhan A, Saleem P, Chinoy R. Papanicolaou stain: is it economical to switch to Rapid economical, acetic acid, Papanicolaou stain. Acta Cytol 2006;50:643–6.·
[12]. Gachie RN, Muchiri WL, Ndungu JR. A comparison of modified and standard papanicolaou staining methods in the assessment of cervical smears at Kenyatta national hospital. East African Medical Journal.2011;88 (7):244-250 .

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