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A COMPARISON OF MODIFIED AND STANDARD PAPANICOLAOU STAINING METHODS IN THE ASSESSMENT OF CERVICAL SMEARS AT KENYATTA NATIONAL HOSPITAL
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R. N. GACHIE, L. W. MUCHIRI and J. R. NDUNGU

ABSTRACT

***Background*: Invasive carcinoma of the cervix is one of the most common cancers in the world and it is preventable by screening methods. The traditional screening method is the Pap smear. As practiced conventionally Pap stain is expensive utilising a considerable amount of alcohol and consumes a lot of time.**
***Objective*: To compare modified and standard Papanicolaou (Pap) staining methods in the assessment of the cervical smears.**
***Design*: A descriptive cross sectional study.**
***setting*: Kenyatta National Hospital.**
***Subjects*: One hundred and sixty two women who were eligible for a pap smear and met the inclusion criteria.**
***Results*: The study showed that the modified Papanicolaou method took three minutes and the standard Papanicolaou method took 20 minutes to stain each bunch of slides. The cost of alcohol used per smear was Ksh 18.50 and Ksh 123.45 per smear in the modified pap method and standard Papanicolaou method respectively. The staining characteristics in the modified method were better than the standard method and there was no compromise in the diagnosis.**
***Conclusions*: The modified Pap staining method is simple, low cost and better in the staining of the cervical smears and can therefore be used as alternative to the standard Pap method in the screening for cervical cancer.**

INTRODUCTION

Cervical cancer is a major cause of death worldwide, with approximately 490 000 women diagnosed annually with invasive cervical cancer and accounts for over 230 000 deaths annually. The majority of cases (80%) occur in developing countries where it is frequently the second most common cancer from 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). A regular Pap smear provides an opportunity to detect pre-cancerous cells in the cervix (4). Epidemiological data show that annual screening reduces the mortality by 70% and the probability of developing invasive carcinoma is reduced by over 95% (5,6).

Standard Pap smear has been the most successful cancer screening test in history since its introduction in the 1950s (4). As practiced conventionally standard Pap stain use a substantial quantity of alcohol which hinders its use as a mass screening tool in low-resource settings. It is also a complex procedure taking a long time to complete the staining (7).

Papanicolaou stain has undergone various modifications in different laboratories. The original modifications of pap stain (1942) were published by Dr. Papanicolaou in 1954 and 1960 (8, 9).

Recent pap modification studies have been done in order to reduce time, alcohol use and improve the staining quality (10-12). However, no such studies have been conducted or adopted in our local settings. The modified Papanicolaou method was intended to reduce the staining time and the cost of staining without compromising the quality or the cytodiagnosis of the smear.

MATERIALS AND METHODS

This descriptive cross sectional study was done at Kenyatta National Hospital's gynaecology clinic, family planning clinic and cytology laboratory. Kenyatta National Hospital is the largest hospital in Kenya and it is both a referral and a teaching hospital. It is located in the capital city, Nairobi. It essentially serves as a provincial and district hospital for the city of Nairobi.

The inclusion criteria were adult women 18-49 years old and had not had total hysterectomy. And the exclusion criteria were women who had a total hysterectomy. Permission to carry out the study was sought from department of Pathology, University of Nairobi and Kenyatta National Hospital, ethics, research and standards committee before commencement of the study.

A sample size of 162 women was determined statistically and those who met the inclusion criteria were informed of the study and consent was obtained. Those who consented were requested to sign the consent forms and a structured questionnaire was then administered to all participants. The demographic data which includes the age, LMP, number of sexual partners, age at first sexual contact were obtained. Clinical history and results of pelvic (cervix) examination was obtained and recorded by the nurse/gynaecologist.

A Pap smear was then taken from the cervix using a cervex broom and was dropped into a vial containing 10 -15 ml of Pap spin collection fluid. The liquid based method was used to prepare the smear. The fluid was divided into two and cyto-centrifuged. One split smear was stained by the standard Pap staining procedure which consisted of 25 dishes, of which eight contained 95% alcohol, three absolute alcohols and two contained alcohol-based counter-stains (OG 6 and EA). The other dishes contained water for rinsing among others (Table 1).

Table1

Conventional and modified Papanicoloau staining protocols

Conventional protocol		Modified protocol	
95% alcohol (for fixation)		95% alcohol (for fixation)	
Tap water	10 dips	1% acetic acid	10 dips
Tap water	10 dips		
HarrisHaematoxylin	2 minutes	Harris Haematoxylin preheated 60°C	10 dips
Tap water	10 dips		
Tap water	10 dips		
Scotts Solution	2 minutes		
Tap water	10 dips		
Tap water	10 dips		
95% alcohol	10 dips	1% acetic acid	10 dips
95% alcohol	10 dips		
OG 6	1 minute		
95% alcohol	10 dips		
95% alcohol	10 dips		
95% alcohol	10 dips		
Eosine azure	10 minutes	Eosine Azure	10 dips
95% alcohol	40 dips	1% acetic acid	10 dips
95% alcohol	40 dips		
95% alcohol	20 dips		
100% alcohol	10 dips		
100% alcohol	10 dips		
100% alcohol	10 dips	95% alcohol	10 dips
Xylene	10 dips		
Xylene	10 dips		
Xylene	10 dips		
Xylene	10 dips		
DPX mount	Coverslip	DPX mount Coverslip	

The other smear was stained by the modified Papaniclaou method, where minimal alcohol was used (only for the initial fixation of smears and prior to mounting). In the rest parts of the procedure alcohol was replaced by 0.5 % acetic acid and also, one alcohol-based counter-stain Orange G was omitted from the procedure because it has been proved to add no diagnostic value of the stain and so its omission does not interfere with the results (13). The haematoxylin used was preheated to 60°C to facilitate stain penetration and hence only a few staining dishes were used as shown in table 1. The smears were coded for each staining method by the assistant who revealed them at the end of data collection procedure. This was done for the purpose of blinding the readers to the staining method used for each case in order to reduce bias for any of the method during reporting.

The two sets of trays were first examined by the investigator for primary examination. The slides were then distributed to two cytopathologists / supervisors for the final screening and reporting who were also blinded to the staining protocol. The reporting was done using The Bethesda System 2001 of reporting cervical smears. The various nuclear and cytoplasmic parameters for staining quality and morphological details were assessed, scored and recorded during the reporting (Tables 2). After the smear reporting was complete, the assistant then revealed the identity of the codes. The smears were then decoded by the investigator and each pair of smears were individually compared and the two protocols analysed to assess the staining characteristics and cytodiagnosis using descriptive statistic and the test of significance was determined.

RESULTS

Out of 162 paired smears examined in the study, 159 pairs were found satisfactory for evaluation. Three pairs of smears were excluded as they were unsatisfactory for evaluation due to scanty cellularity and a repeat was recommended.

The minimum age of the patients screened was 21 years and maximum was 49 years. The majority were between 33-38 years which was (24.1%). The mean age was 37.1, SD of 8.0 and a median of 36.5 (Figure 1).

In the modified Papanicolaou procedure only 1.5 liters 95% (including fixation, stain solution and top ups) in staining 162 smear which costed Ksh 3000 whereas in the standard Papanicolaou procedure, ten litres of alcohol was consumed which costed Ksh 20,000. The staining of each batch of 50 slides could be completed in three to four minutes by modified Papanicolaou method, whereas it took almost 20 minutes by standard Papanicolaou method (Table 2).

The staining quality was compared in both methods and the P-value in the staining of the cytoplasmic borders and nuclear borders shows no statistically significant difference in both methods. In the staining of the cytoplasm and nuclear chromatin the P-value indicated that there was statistically significant difference in both methods (Table 3).

Eighteen (11.3%) of the smears had indistinct cell borders and one hundred and fourty one (87%) had distinct cell borders in the standard pap method. In modified Pap method fifteen (9.5%) of the smears had indistinct cell borders and one hundred and fourty four (90.6%) had distinct cell borders (Figure 2).

Table 2
Quantified cost and time for the two methods

	Modified Pap	Standard Pap
Time (minutes)	3 ± 0.5	20 ± 0.5
Reagents & amount	1.5 liters of alcohoL	
	15 mls of acetic acid	10 liters of Alcohol
Total cost of the alcohol used	Ksh 3000	Ksh 20000
Total cost of the acetic acid used	Ksh 15	0
Cost/Pap smear for alcohol	KSH 18.50	KSH 123.45
Cost/pap smear for Acetic acid	90 cents	
Cumulative cost per slide	Ksh 19.40	Ksh 123.45

The standard Papanicolaou method had fourteen (8.8%) unsatisfactory cytoplasmic staining, one hundred and two (64.2%) satisfactory and fourty three (27.0%) were excellent. Modified Papanicolaou method had ten (6.3%) in the unsatisfactory cytoplasmic staining, fifty three (33.3%) satisfactory and ninety six (60.4%) stained excellently (Figure 3).

The nuclear membranes/borders were well stained in both methods with just a slight variation. In SP 95.6% nuclear borders were distinct and 4.4% were indistinct. Ninety six point nine percent were distinct and 3.1% indistinct in the modified Papanicolaou (Figure 4).

In the standard Papanicolaou the chromatin staining was hazy in seventy nine (49.7%) and distinct in eighty (50.33%), while in modified Pap chromatin staining was hazy in fifty four (34%) and distinct in one hundred and five (66%) in standard Papanicolaou (Figure 5).

In this study the diagnoses concurred in all cases in the paired smears and the pathologists/cytologists encountered no difficulties in the interpretation of the smears stained by the modified Pap. There were one hundred and fifty two (95.6%) negative smears for intraepithelial lesion or malignancy, and seven (4.4%) epithelial cell abnormalities (ASCUS, LSIL and HSIL). 1.25%, 1.89% and 1.25% were ASCUS, LSIL and HSIL respectively. Inflammatory and reactive changes were ninety six (60.37%) (Table 3).

Table 3
Cytomorphologic features

Parameter	Standard Pap	%	Modified Pap	%	P-Value
1.Cell/Cytoplasmic borders					
Indistinct	18	11.3	15	9.4	
Distinct	141	88.7	144	90.6	X^2(0.30),P=0.71
2.Cytoplasmic Staining					
Unsatisfactory	14	8.8	10	6.3	
Satisfactory	102	64.2	53	33.3	
Excellent	43	27.0	96	60.4	X^2(36.4),P=0.00 <0.05
3.Nuclear borders					
Indistinct	7	4.4	5	3.1	
Distinct	152	95.6	154	96.9	X^2(0.35),P=0.77
4.Chromatin staining					
Hazy	79	49.7	54	34.0	
Distinct	80	50.3	105	66.0	X^2(8.08),P=0.006 <0.05

Figure 1
Age distribution of the women screened for cervical cancer

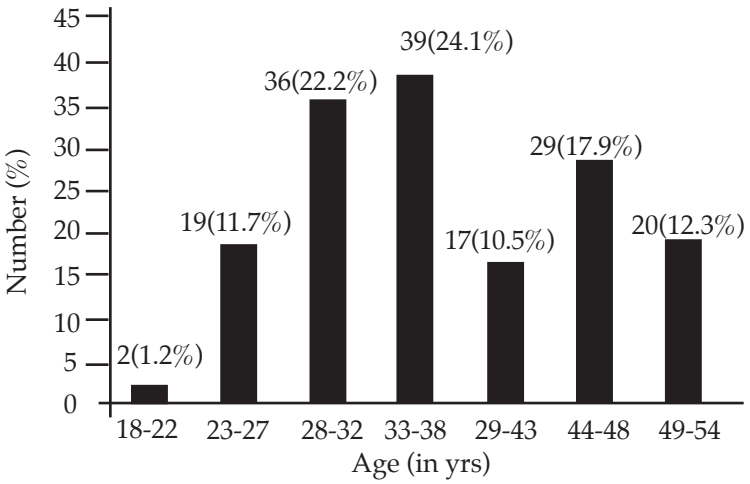


Figure 2
Staining of the Cell borders

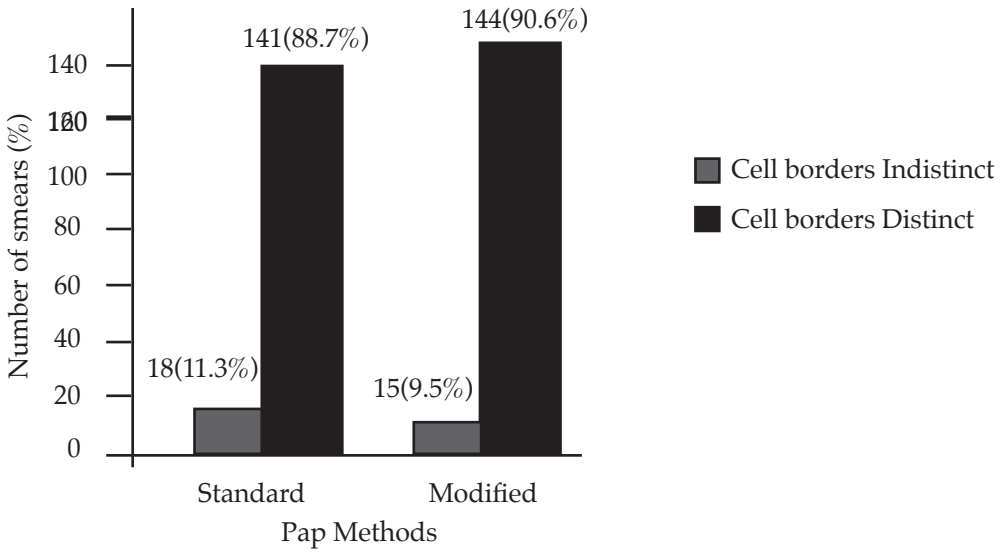


Figure 3
Cytoplasmic Staining

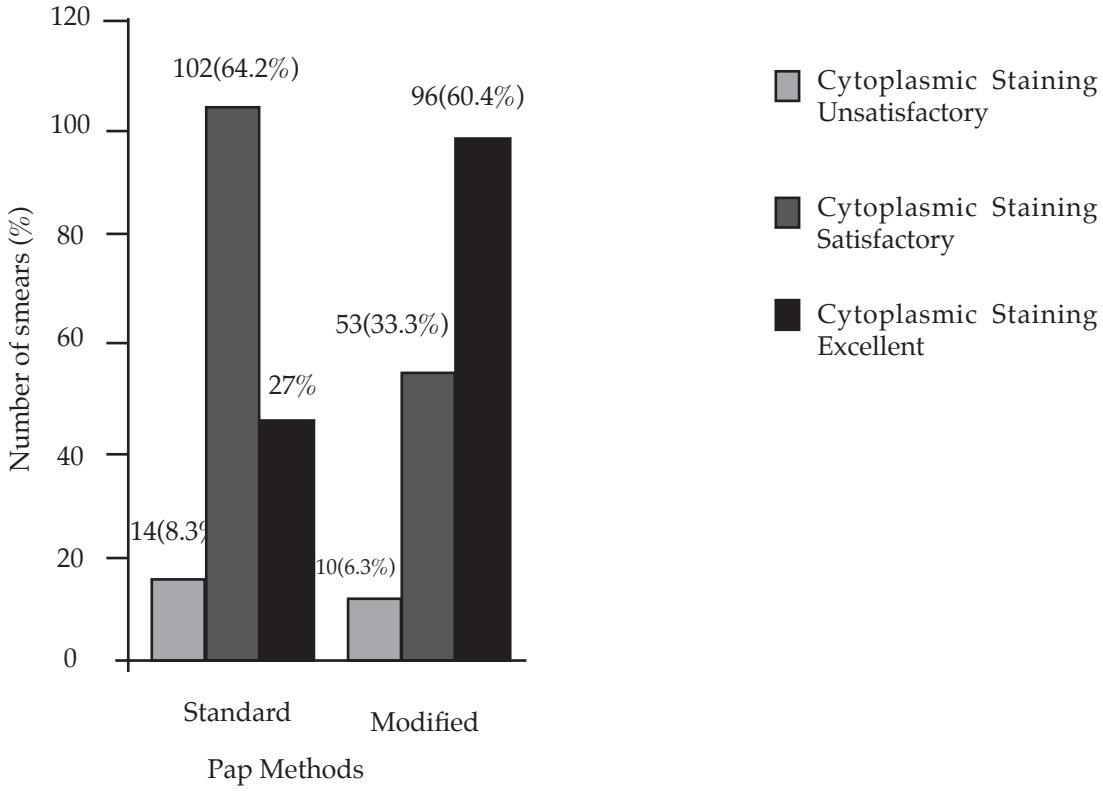


Figure 4
Staining of nuclear borders

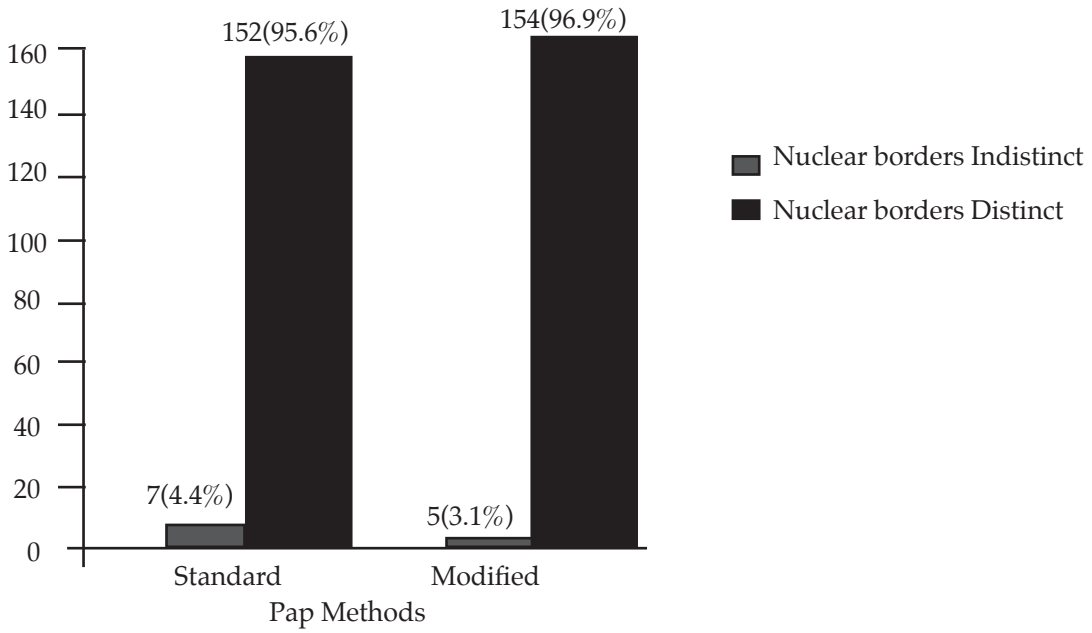
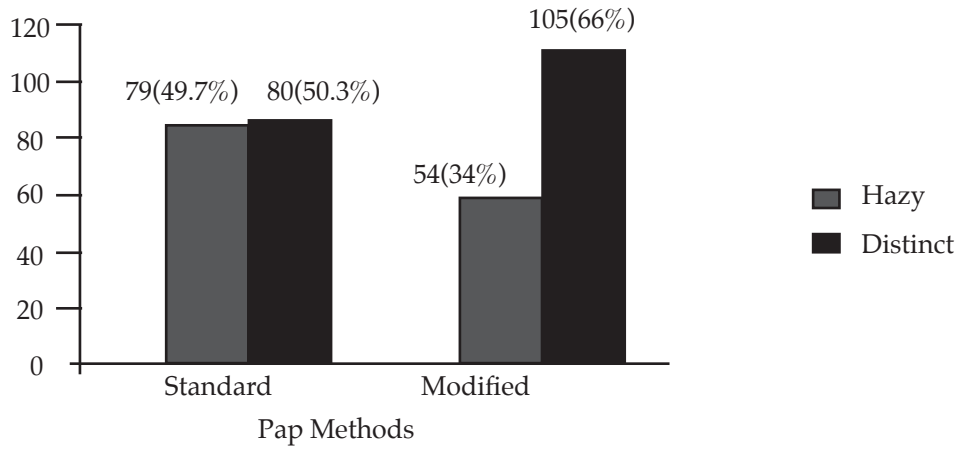


Figure 5
Chromatin staining (appearance)



DISCUSSION

The Pap smear has been utilised for cervical cancer screening for more than 50 years. Pap smear procedure are complex and costly to run and have failed to reach a significant proportion of women (14). The conventionally practiced method (standard Papanicolaou) is complex, with multiple steps of greatly varying times, making it liable to laboratory errors. As a result of above reasons Papanicolaou staining method has been modified in several ways (4).

This study was to compare the modified Papanicolaou and the standard Papanicolaou methods in the screening of the cervical smears. The modified Papanicolaou sought to improvise on standard Papanicolaou by replacing the alcohol with 0.5 % acetic acid in all the steps except the initial fixation and prior to mounting the smears.

The participants in the study were 162 of whom 159 smears were satisfactory for evaluation and three were unsatisfactory where a repeat was recommended.

The ages of the women who participated in the study ranged between 22-49 years, majority being in the ages 33-38 years. The mean age of the participant was 37.1, standard deviation of 8.0 and a median of 36.5 years. On Papanicolaou screening only 34.5% of the women had ever had Pap test and 91.4% of these Pap smears were normal. This is slightly lower than in a study done by Gatune *et al.*, (15) at Tigoni, Kenya who found out that 67% of those screened had had a pap smear. The mean age of those who were screened was the same that is 37.1.

The study showed that the time taken to stain each bunch Pap smear slides (25 slides, 40 slides depending on the slide carrier) in the modified Papanicolaou method was reduced considerably to 3 ± 0.5 minutes while in the standard Papanicolaou method the time taken was 20 ± 0.5 minutes for the same number of slides. This concurred with a study by Gupta *et al*., (7) for both standard Papanicolaou method and modified Papanicolaou staining time.

In the modified Papanicolaou procedure the cost of alcohol which was consumed was Ksh 2000 and the cost in the standard method was Ksh 20000. The cost of alcohol per smear in the modified Papanicolaou method was Ksh 18.50 and 123.45 per smear in the standard method. Papanicolaou. This shows a drastic reduction of 1/7 of the total cost of staining in the modified pap compared to standard Pap method. This is comparable to a study done by Gupta *et al* (7) which also showed that it took three to four minutes to stain a batch of 50 slides and a reduction of 1/6 of the total cost in modified Papanicolaou compared to Standard method.

The simplicity of the procedure (uniform 10 dips at each step) also reduced the risk of errors while staining because there is no variation of time or dips from one staining container to the other unlike in the standard Pap where each container has different timings during staining.

The staining quality of both nuclear chromatin and cytoplasm in our modified protocol was found to be better than the standard Pap protocol but the diagnosis in the two methods was the same.

In conclusion, the modified Papanicolaou staining procedure with minimum alcohol use is a simple and technician friendly protocol that does not compromise on staining quality and diagnostic standards. It can be easily adopted as a suitable alternative to the expensive and time consuming standard protocol for mass screening of cervical cancer in limited resource settings.

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