Original Article

Comparative Study of Modified Ultrafast Papanicolaou and Standard Papanicolaou Staining Technique for the Assessment of Cervical Smears

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Abstract

Introduction: Cervical invasive carcinoma is one of the most frequent cancers in the world, and it can be prevented with screening. The papanicolaou (Pap) smear is the most common screening procedure. Pap staining, as it is now done, is costly, requires a large amount of alcohol, and takes a long time. **Materials and Methods:** A total of 170 patients were recruited. One hundred and sixty-six patients, considering eligibility criteria, undergoing Pap smear test. Eligibility criteria: Age: 18–49 years females, who did not have a total hysterectomy done, who consented to participate in the study. Each patient had two smears taken on glass slides. Standard Pap smears were fixed for at least 15 min in 95% ethanol. The other smear was air-dried before being fixed with alcohol formalin and stained with modified ultrafast Pap (MUFP) stain. A comparison of two different techniques was made on the basis of cytomorphological features. **Results:** In studied smears, a clean background was seen in 18.24% and 14.12% of the cases in Pap stain and MUFP, respectively. Kappa analysis found a good correlation between Pap stain and MUFP (kappa: 0.81, $P < 0.01$). Crisp chromatin was seen in 89.41% and 67.65% of MUFP and Pap staining, respectively. About 88.82% of MUFP stains showed optimal cytoplasmic details and the same was revealed by 81.18% of Pap stains. MUFP has a better quality index (QI) as compared to Pap staining as no case in MUFP had QI of < 0.80 with a statistically significant difference as $P < 0.05$. **Conclusion:** MUFP is a simple, user-friendly, affordable, and less time-consuming alternative in low-resource areas in comparison to the traditional technique for mass cervical cancer screening.

Keywords: Cervix, modified ultrafast, papanicolaou smear, papanicolaou

Introduction

Worldwide, with over 490,000 people diagnosed each year and over 230,000 deaths due to cervical cancer (CC).[1] In developed countries, there is around 80% of occurrences after the prevalent malignancy breast cancer.[2] The lesions are precancerous that appear gradually over time.[2]

Every year, CC affects 122,844 Indian women, resulting in 67,477 deaths. CC is a threat for 40 million Indian women between 15 and 64 years. It is the second most prevalent malignancy among women aged 15–44 years old. Among the Asian countries, CC was found to be highest in the Indian population (22%). As CC is the second-most common malignancy, understanding its epidemiology is crucial.[3]

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CC can be prevented, and there are screening methods for detecting it at an early stage. One such trustworthy approach is a papanicolaou (Pap) smear based on cytology. Precancerous cells in the cervix can be detected with a regular Pap smear. Annual screening reduces death by 70% and the risk of developing invasive cancer by over 95%, according to epidemiological study.[4,5]

For both gynecological and nongynecological cytology smears, Pap stain is the best stain. The Pap stain produces a polychromatic

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staining reaction that is transparent and has distinct nuclear and cytoplasmic characteristics. Dr. George Nicholas Papanicolaou developed this stain in 1942 to determine changes in cellular maturity and metabolic function in vaginal smears. The Pap stain distinguishes between basophilic and acidophilic cell components and creates a precise chromatin pattern, allowing for thorough nuclear examination.[6,7]

The Pap stain has evolved significantly throughout time. The necessity for a rapid approach for analyzing cervical Pap smears with a short turnaround time has led to developments in staining procedures that require less staining time while ensuring equivocal cell morphology. The ultrafast papanicolaou (UFP) stain was created by Yang and Alvarez in 1995.[8] UFP stain is a hybrid of Romanowsky's technique and regular Pap stain that takes <2 min to stain.[9]

Although almost every compound used in UFP is commonly available, Kamal *et al*.[10] changed the process by replacing Richard-Allan's hematoxylin with Gill's hematoxylin. Air drying of cells, rehydration in natural saline, and fixation in alcoholic formalin are all the steps followed by UFP. The purpose of air drying is to make the cells seem larger, which improves cellular analysis resolution. The cells are rehydrated with normal saline, then alcoholic formalin (pH 5) is used to bring out the rich colors in the cells and the nucleoli, which stain red, in addition to the background blood's hemolysis. Much of this may be done in <90 s. The whole process is quick enough to allow microscopic examination of the cytological smears right away.[10]

The staining solutions used in Yang and Alvarez's UFP method were commercial preparations, which is a disadvantage. Richard Allan hematoxylin and Cyto-Stain are produced by Richard-Allan, Inc. (Richland, Michigan, USA), and are not widely available. Harris hematoxylin was used in this investigation instead of Gill's hematoxylin since it is more readily available.[11]

The main motive for modifying Pap stain is to reduce turnaround time and speed up reporting, which saves time, reduces alcohol use, and improves staining efficiency without sacrificing Pap stain cytodiagnosis quality. However, no such research based on this has been conducted in our area to date. The necessity for Pap stain alterations stems from the requirement to examine or monitor the cervical Pap smear in a short period to avoid or limit the occurrence of invasive cervical malignancy. This study was conducted to compare the efficacy of the modified ultrafast Pap (MUFP) Technique as well as to define the SP Technique for Cervical Smear Assessment.

Materials and Methods

Study design

This is a prospective study conducted in the Department of Pathology at TMMC and RC Moradabad.

Study setting

The study was conducted from December 2019 to November 2021 and was approved by the institutional ethics committee (TMMC and RC/19-20/054).

Table 1: Material and method

Standard Pap staining[10]	Time	Modified ultrafast Pap stain[10]	Time
95% alcohol (fixation)	10 min	Air dried smear	30 s
80% alcohol	10 s	Normal saline	3 dip
70% alcohol	10 s	Alcohol formalin	10 s
50% alcohol	10 s	Tap water	6 dip
Tap water	3 min	Harris hematoxylin	30 s
Harris hematoxylin	5 min	Tap water	6 dip
Tap water	3 min	95% alcohol	6 dip
1% acid alcohol	1 dip	Modified EA_{36}	15 s
Tap water	5 min	95% alcohol	6 dip
OG_6	4 min	Xylene	10 dip
95% alcohol	30 dip	DPX mount	Coverslip
EA-36	10 min		
95% alcohol	80 dip		
100% alcohol	30 dip		
Xylene	30 dip		
DPX mount	Coverslip		

PAP: Papanicolaou, DPX: Dibutylphthalate Polystyrene Xylene, EA-36: Eosin Azure 36, OG-6: Orange G-6

Table 2: Scoring system used in assessment of staining[15]

Parameter	Score=1	Score=2	Score=3
Background	Hemorrhage	Clean	-
Overall staining	Poor	Average	Good
Cell morphology	Poorly preserved	Moderately preserved	Well preserved
Nuclear characteristics	Smudgy chromatin	Moderately crisp	Crisp chromatin
Cytoplasmic details	Unsatisfactory	Suboptimal	Optimal
Air drying artifacts (%)	>50	<50	0

Sample size

A total of 170 patients were recruited. One hundred and sixty-six patients, considering eligibility criteria, undergoing Pap smear test in the department of pathology, TMMC, and RC during the study period were included in the study.

Patients conforming to eligibility criteria were taken. A thorough history which includes demographic data such as age and last menstrual periods. The detailed clinical and cervical examination was performed by the nurse/gynecologist. Each patient had two smears taken on glass slides. One of the smears was fixed for at least 15 min in 95% ethanol. Standard Pap staining was used on these smears as shown in Table 1.

The other smear was air dried before being fixed with alcohol formalin and stained with MUFP stain as shown in Table 1. Protocol for routine Pap staining and modified Pap staining techniques was followed. Comparison of two different techniques was made on the basis of cytomorphological features.

The "Quality Index (QI)" was calculated as the ratio of the actual score obtained to the maximum score possible as shown in Table 2.

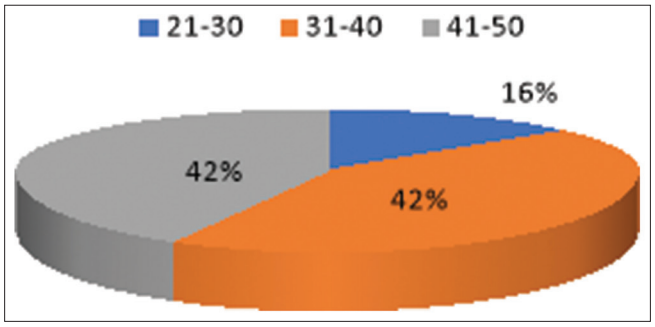
QI = actual score obtained/maximum score.[8]

Results

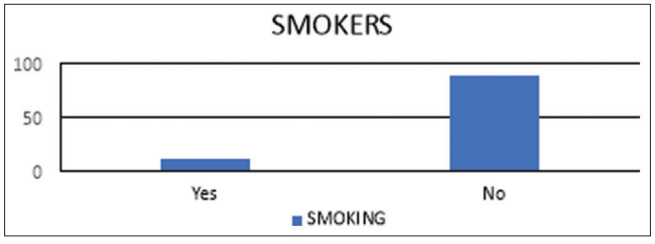
The present cross-sectional study was done in the Department of Pathology, TMMC, and RC among 170 patients who underwent Pap smear test in the Obstetrics and Gynecology outpatient department included.

Out of 170 subjects, 27 (15.88%), 72 (42.35%), and 71 (41.76%) of the subjects were from 21–30, 31–40, and 41-50 years age group, respectively [Table 3 and Graph 1].

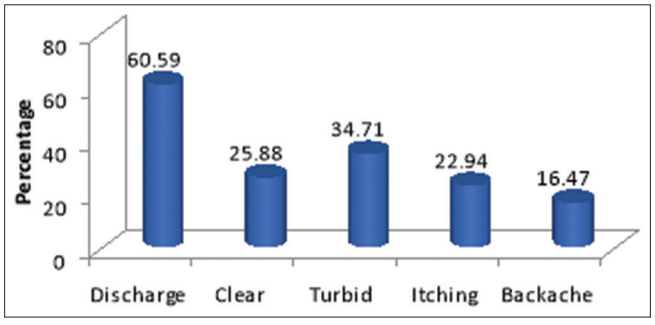
Only 19 patients (11.18%) gave a history of smoking [Table 4 and Graph 2]. The most common clinical feature among the study subjects was vaginal discharge 107 (60.59%) followed by itching 39 (22.94%) and backache 28 (16.47%). Clear and turbid discharge was found in 44 (25.88%) and 59 (34.71%) of the subjects, respectively [Table 5 and Graph 3].



Graph 1: Age distribution among the study subjects



Graph 2: Smoking habit distribution among the study subjects



Graph 3: Presenting clinical symptoms among the study subjects

The clean background was seen in 139 (81.76%) Pap-stained smears and 146 (85.88%) MUFP-stained smears, respectively. The hemorrhagic background was present in 18.24% and 14.12% of the cases in Pap stain and MUFP, respectively. Kappa analysis found a good correlation between Pap stain and MUFP (kappa: 0.81, $P < 0.01$), as shown in Table 6 and Graph 4.

One hundred and forty-four (84.71%) MUFP and 110 (64.71%) Pap-stained smears showed an overall good staining pattern. Average staining was observed in 60 (35.29%) Pap and 26 (15.29%) MUFP-stained smears, respectively. Hence, there was no good correlation between MUFP and Pap staining [Table 7 and Graph 5].

Well-preserved cell morphology was seen in 145 (85.29%) MUFP-stained smears and 122 (71.76%) Pap-stained smears, respectively. Although Pap staining revealed less-well-preserved morphology in comparison to MUFP stain, still Kappa analysis found a good correlation between Pap stain and MUFP (kappa: 0.73, $P = 0.037$), as shown in Table 8 and Graph 6.

Table 3: Age distribution among the study subjects

Age group (years)	Number of patients (%)
21–30	27 (15.88)
31–40	72 (42.35)
41–50	71 (41.76)
Total	170 (100)

Table 4: Distribution of patients according to their smoking habit

Smokers	Number of patients (%)
Yes	19 (11.18)
No	151 (88.82)

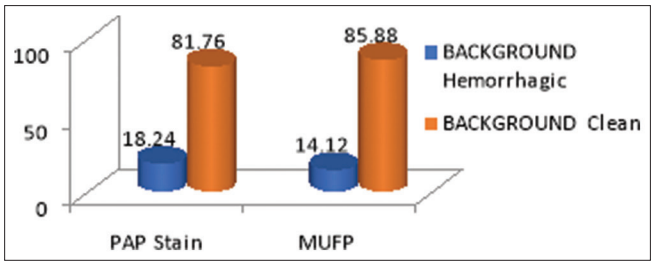
Table 5: Presenting clinical symptoms among the study subjects

Variables	Number of patients (%)
Vaginal discharge	103 (60.59)
Clear	44 (25.88)
Turbid	59 (34.71)
Itching	39 (22.94
Backache	28 (22.94)
Total	170 (100)

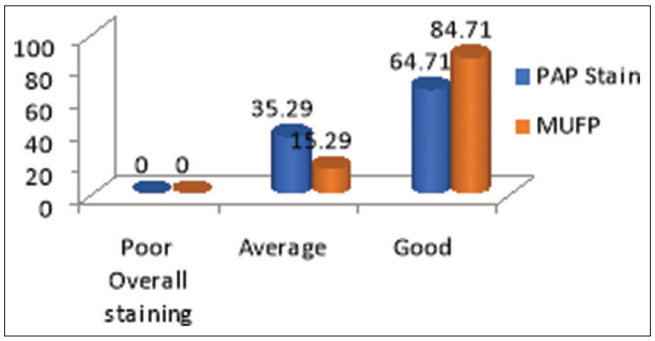
Table 6: Background comparison among the Papanicolaou stain and modified ultrafast papanicolaou

Background	Pap stain (n=170), n (%)	MUFP (n=170), n (%)	Kappa value	P
Hemorrhagic	31 (18.24)	24 (14.12)	0.81	<0.01*
Clean	139 (81.76)	146 (85.88)		

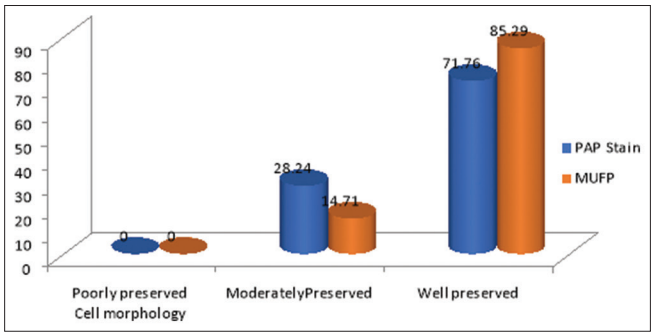
*Statistically significant. PAP: Papanicolaou, MUFP: Modified ultrafast PAP



Graph 4: Background comparison among the papanicolaou (PAP) stain and modified ultrafast PAP. PAP: Papanicolaou, MUFP: Modified ultrafast PAP



Graph 5: Overall staining comparison among the papanicolaou (PAP) stain and modified ultrafast PAP. PAP: Papanicolaou, MUFP: Modified ultrafast PAP



Graph 6: Cell morphology comparison among the papanicolaou (PAP) stain and modified ultrafast PAP. PAP: Papanicolaou, MUFP: Modified ultrafast PAP

Crisp chromatin was seen in 152 (89.41%) MUFP-stained smears and 115 (67.65%) Pap-stained smears, respectively. Hence, Pap staining showed less crisp chromatin as compared to MUFP stain. Kappa analysis also found an average correlation between Pap stain and MUFP (kappa: 0.68, $P = 0.06$) as shown in Table 9 and Graph 7.

One hundred and fifty-one (88.82%) MUFP-stained smears showed optimal cytoplasmic details and almost similar cytoplasmic detail 138 (81.18%) was revealed by Pap-stained smears. Thirty-two (18.82%) Pap-stained smears and 19 (11.18%) MUFP smears showed suboptimal cytoplasmic details, as shown in Table 10 and Graph 8. Kappa analysis showed a good correlation between Pap stain and MUFP stain (kappa: 0.79, $P = 0.024$).

Table 7: Overall staining comparison among the papanicolaou stain and modified ultrafast papanicolaou

Overall staining	Pap stain ($n=170$), n (%)	MUFP ($n=170$), n (%)	Kappa value	P
Poor	0	0	0.63	0.09
Average	60 (35.29)	26 (15.29)		
Good	110 (64.71)	144 (84.71)		

PAP: Papanicolaou, MUFP: Modified ultrafast PAP

Table 8: Comparison of cytological details preservation among the papanicolaou and modified ultrafast papanicolaou stain

Cell morphology	Pap stain ($n=170$), n (%)	MUFP stain ($n=170$), n (%)	Kappa value	P
Poorly preserved	0	0	0.73	0.037*
Moderately preserved	48 (28.24)	25 (14.71)		
Well preserved	122 (71.76)	145 (85.29)		

*Statistically significant. PAP: Papanicolaou, MUFP: Modified ultrafast PAP

Table 9: Nuclear characteristics comparison among the papanicolaou stain and modified ultrafast papanicolaou stain

Nuclear characteristics	Pap stain ($n=170$), n (%)	MUFP ($n=170$), n (%)	Kappa value	P
Smudgy chromatin	0	0	0.68	0.06
Moderately crisp chromatin	55 (32.35)	18 (10.59)		
Crisp chromatin	115 (67.65)	152 (89.41)		

PAP: Papanicolaou, MUFP: Modified ultrafast PAP

Table 10: Cytoplasmic details comparison among the papanicolaou stain and modified ultrafast papanicolaou stain

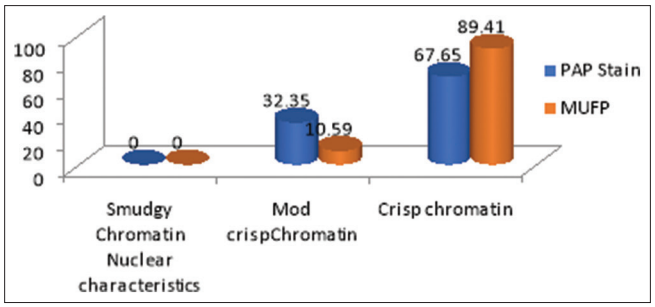
Cytoplasmic details	Pap stain ($n=170$), n (%)	MUFP ($n=170$), n (%)	Kappa value	P
Unsatisfactory	0	0	0.79	0.024*
Sub-optimal	32 (18.82)	19 (11.18)		
Optimal	138 (81.18)	151 (88.82)		

*Statistically significant. PAP: Papanicolaou, MUFP: Modified ultrafast PAP

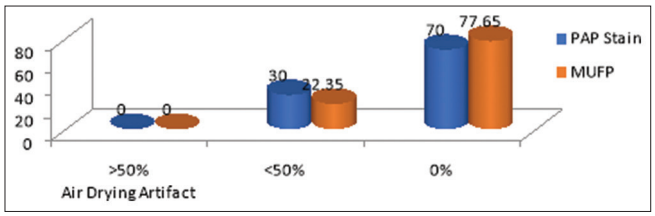
MUFP-stained smears showed 38 (22.35%) comparatively less air-drying artifacts as compared to Pap stain, 51 (30%), as shown in Table 11 and Graph 9. QI was better in MUFP as compared to Pap staining, as no case in MUFP had QI of < 0.80. When QI was compared statistically between MUFP and Pap staining, a significant difference was found as $P < 0.05$ [Table 12 and Graph 10].

Discussion

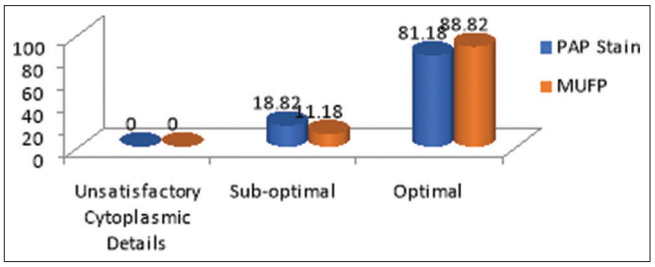
This study was to compare the standard Pap stain with the



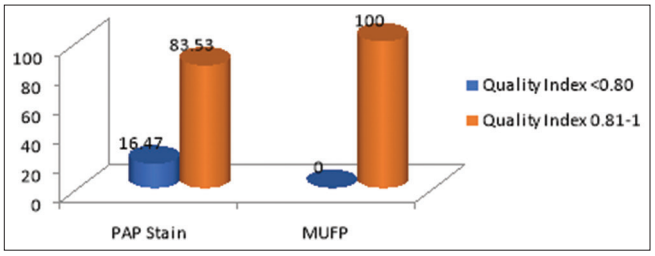
Graph 7: Nuclear characteristics comparison among the papanicolaou (PAP) stain and modified ultrafast PAP stain. PAP: Papanicolaou, MUFP: Modified ultrafast PAP



Graph 9: Air drying artifact comparison among the papanicolaou (PAP) and modified ultrafast PAP stain. PAP: Papanicolaou, MUFP: Modified ultrafast PAP



Graph 8: Cytoplasmic details comparison among the papanicolaou (PAP) Stain and modified ultrafast PAP stain. PAP: Papanicolaou, MUFP: Modified ultrafast PAP



Graph 10: Quality index comparison among the papanicolaou (PAP) stain and modified ultrafast PAP stain. PAP: Papanicolaou, MUFP: Modified ultrafast PAP

MUFP staining technique in terms of its time consumption, cost-effectiveness, and superiority in staining quality in making the diagnosis or assessing the cervical smears.

Out of 170 subjects, majority of the case subjects were between the age of 31 and 40 years in our study, which is comparable to Pudasaini *et al*.[11] as their study revealed a mean age of 36.57 years and Gachie *et al*.[12] in their study found that the majority of the subjects were between 33 and 38 years.

Maximum patients gave a negative history of smoking, alcohol consumption, or any kind of addiction and only 19 patients (11.18%) gave a history of smoking in our study.

In the present study, the most common presenting clinical symptom was vaginal discharge 107 (60.59%), followed by itching 39 (22.94%) and backache 28 (16.47%) of the subjects, respectively. Similar to the study of Verma *et al*.[13] as in their study found that the most common presenting complaint was abnormal vaginal discharge 54.5% along with intermenstrual bleeding 19.5%.

The clean background was seen in 31 (18.24%) and 24 (14.12%) of the cases in Pap stain and MUFP stain, respectively. The hemorrhagic background was revealed in 139 cases (81.76%) and 146 (85.88%) of the cases in Pap stain and MUFP, respectively. Kappa analysis found a good correlation between Pap stain and MUFP (kappa: 0.81, $P < 0.01$) which is comparable to the study of Pudasaini *et al*.[11] which showed that there was a slight difference in background between UFP and standard Pap stain when the smear was hemorrhagic and inflammatory, Choudhary *et al*.[8] who discovered that UFP-stained smears had a clean red blood cell-free background in their experiments.

Table 11: Air drying artifact comparison among the papanicolaou stain and modified ultrafast papanicolaou stain

Air drying artifact (%)	Pap stain (n=170), n (%)	MUFP (n=170), n (%)	Kappa value	P
>50	0	0	0.74	0.031*
<50	51 (30)	38 (22.35)		
0	119 (70)	132 (77.65)		

*Statistically significant. PAP: Papanicolaou, MUFP: Modified ultrafast PAP

Table 12: Quality index comparison among the papanicolaou stain and modified ultrafast papanicolaou stain

QI	Pap stain (n=170), n (%)	MUFP (n=170), n (%)	χ^2	P
<0.80	28 (16.47)	0	6.81	0.017*
0.81–1	142 (83.53)	170 (100)		

*Statistically significant. QI: Quality index, PAP: Papanicolaou, MUFP: Modified ultrafast PAP

Although background, cell morphology, and overall staining were better in MUFP-stained smears than in traditional Pap smears, the difference in staining was not statistically significant, according to Kannan *et al*.[14]

One hundred and forty-four (84.71%) and 110 (64.71%) MUFP and Pap stain cases showed overall good staining quality, respectively. Average staining was revealed in 60 (35.29%) and 26 (15.29%) of Pap and MUFP, respectively. Hence, there was not a good correlation between MUFP and Pap staining.

This correlates very well with other studies, namely Thakur and Guttikonda[15] and Alwahaibi *et al*.[16]

The majority of cases shows well-cell morphology, though conventional Pap staining revealed comparatively less well-preserved morphology as compared to MUFP, still, Kappa analysis found a good correlation between Pap stain and MUFP (kappa: 0.73, $P = 0.037$). This correlates very well with other studies, namely Thakur and Guttikonda[15] and Alwahaibi *et al*.[16] and Pudasaini *et al*.[11] their study showed that MUFP has better well-preserved cell morphology as compared to PAP.

Majorly Crisp chromatin was seen in MUFP as compared to conventional pap. Hence, Pap staining revealed less crisp chromatin as compared to MUFP and Kappa analysis also found an average correlation between Pap stain and MUFP (kappa: 0.68, $P = 0.06$). Similar to R. N. Gachie *et al*.[12] in their study showed that nuclear chromatin the modified Pap protocol outperformed the regular Pap protocol.

One hundred and fifty-one (88.82%) MUFP-stained smears displayed optimum cytoplasmic features, while 138 (81.18%) Pap-stained smears demonstrated the same. Thirty-two (18.82%) and 19 (11.18%) of the Pap and MUFP cases showed suboptimal cytoplasmic details. Kappa analysis found a good correlation between Pap stain and MUFP (kappa: 0.79, $P = 0.024$). Similarly, Gachie *et al*.[12] discovered finding cytoplasmic on their modified Pap technique was better than the normal Pap protocol in their investigation.

MUFP (22.35%) showed comparatively less air-drying artifacts as compared to Pap (30%). MUFP is better than Pap staining as no case in MUFP had QI of <0.80. When QI was compared statistically between MUFP and Pap staining found significant $P < 0.05$. Similar to the study of Kannan *et al*.[14] found that the QI of MUFP was higher than that of conventional Pap stain in a study.

Hence, it can be said that the advantages of MUFP over Pap stain were a clean background, no fixation time required, and usefulness for intraoperative cytological analysis. Sample adequacy can be assessed rapidly, and cell loss due to wet fixation can be avoided with precise sample collection. Furthermore, the advantage of MUFP over Pap is the combination of air drying and fixation technique, which offers good background staining and enlarged nuclei with prominent nuclear features. Therefore, MUFP may be considered a suitable alternative to standard or conventional Pap stain for cervical screening programs.

Conclusion

Most of the subjects had age >30 years. MUFP showed comparatively less air-drying artifacts as compared to PAP. MUFP has a better QI as compared to Pap staining as no case in MUFP had QI of <0.80 with a statistically significant difference as $P < 0.05$.

Finally, MUFP staining with minimum alcohol is a simple and user-friendly process which will neither impact stain accuracy nor diagnosis requirements. In low-resource contexts, it can be easily adapted as an affordable and less time-consuming alternative to the traditional technique for mass CC screening.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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