



Comparison of bloody pap smear fixation by Carnoy's and fixator spray in samples from women with abnormal uterine bleeding

**Keshavarz Najmeh¹, Abdali Khadijeh*², Akbarzadeh Mojgan³, Amoei Sedighe⁴,
Tabatabaei Sayyed Hamidreza⁵ and Dowran Paria⁶**

¹Department of Midwifery, Fatemeh College of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran

²Department of Midwifery, Fatemeh College of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran

³Department of Pathology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

⁴Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

⁵Department of Epidemiology, Health and Nutrition Faculty, Shiraz University of Medical Sciences, Shiraz, Iran

⁶Department of Midwifery, Fatemeh College of Nursing and Midwifery, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding Email: abdali_kh@yahoo.com

ABSTRACT

Abnormal uterine bleeding is a serious problem among women of late reproductive age. Cervical cancer is one of the reasons of AUB and Pap smear is the best way to diagnose it. Blood has negative effects on Pap smear especially in AUB with great blood. One of the effective solutions for lysing RBC is Carnoy's. This study aims to compare two methods of fixation in bloody Pap smear by Carnoy's and normal spray in samples from women with AUB. This study was done on 204 bloody Pap smear from 102 women with AUB that referred to Zeinabieh and Faghihi hospitals of Shiraz University of Medical Sciences from 2012-2013. After observing uterine bleeding in each subject, two samples were provided using usual Pap smear method; one of the slides was fixed by normal spray and the other slide fixed by Carnoy's solution for 20 minutes. After staining (Papanicolaou method), two pathologists performed a double-blind trial to analyze them. Data analysis was done using SPSS. 82 samples did not have cell adequacy. Squamous cells in Carnoy's-fixed slides were more than spray-fixed ones ($p=0.024$). The decrease in the number of RBC on slides and the increase in the clarity of slides in Carnoy's-fixed samples were more than spray-fixed slides ($p=0.001$), while identifying inflammatory cells ($p=0.832$) and microbial factors ($p=1$) in both methods showed no significant difference. Carnoy's solution can be used as an effective fixative in bloody Pap smear from women with abnormal uterine bleeding.

Keywords: uterine bleeding, Papanicolaou Smear, Cervical cancer, fixation

INTRODUCTION

Abnormal uterine bleeding is a prevalent and serious problem among women of late reproductive age [1]. Its causes include the following factors: being in menopause ages, lack of ovulation, using exogenous estrogens, endometritis, atrophic vaginitis, having cervical cancer[2]. Bleeding due to pathologic causes among women of these ages is more than young women. The evaluation of abnormal uterine bleeding includes taking the patient's medical history, physical examination, cytology analysis (Pap smear), endometrial biopsy[3]. As mentioned before, cervical cancer is one of the main causes of uterine bleeding that is currently best diagnosed by taking a Pap smear. During the past six decades, Pap smear has been a screening method in pre-cancer and cervical cancer debris resulted in the reduction of cervical cancer mortality to about 70% [4]. In one of the study of the endometrial carcinoma, the sensitivity of Pap smear in identifying endometrial carcinoma was reported to be 28% [5]. A good Pap smear should contain sufficient squamous and endocervical cells and also provide a good cytology sample from transitional zone and endocervix [6]. Bloody cervical cells are considered as one of the prevalent cytology errors that can interfere in the interpretation of Pap smear results [7].

In Bentz et al's study (2002); bloody smears had composed 12.7% of unsatisfactory Pap smears by thin prep method [8]. In Jamali Zavvareh et al's study (2007) in Tehran's Imam Khomeini Hospital, red globules existed in 7.7% of Pap smear samples [9]. In Ghasemi et al's study (2006), 11% of clients had bleeding at the time of sampling [10]. In another study by Lotfinezhad et al (2002), 10.7% of unsatisfactory Pap smears were covered with blood [11].

According to the mentioned results, bloody smears are considered as one of the most important cytological challenges especially in AUB in which the great amount of blood prevents taking a Pap smear and causes the patient to miss follow-up and treatment process. Fixation is one of the remarkable aspects of histology and its correct implementation allows cytologists and pathologists to observe cellular changes faster and easier. Different solutions are used for fixation and cleaning blood from Pap smear samples such as acetic acid, liquid media and cytorich-red, while each of them has its own specific defects including reduction of the clarity of cells and thrombus formation. One of the most effective solutions in the hemolysis of red globules is Carnoy's solution (ethanol, chloroform, acetic acid, 6:3:1) [12]. To have a better investigation, Carnoy's solution can be used to clean blood from smears before staining the sample. Carnoy's solution is also used to fix other tissues including stomach, esophagus, thyroid and breast. In Shamsi's study, it was used in cytology in order to clean bloody samples of Pap smear that were covered with blood due to contact bleeding (little blood). The present study aims to compare two methods of fixation of Bloody Pap smear slides by Carnoy's solution and normal fixative spray in samples from women with abnormal uterine bleeding.

Materials and Methods: This experimental study was done during 2012-2013 in Women's Health Zeinabieh and Shahid Faghihi Hospitals of Shiraz University of Medical Sciences. 204 bloody Pap smear slides from the cervical cells of 102 women with abnormal uterine bleeding who had referred to the hospital and had bleeding at the time of sampling were studied. The sample size was determined according to the clarity of slides in spray-fixed and Carnoy's-fixed methods with a difference of 18% between the two methods with a statistical power of 80% and a 95% confidence interval. The entire slides were provided from cervical cells that were covered with blood during sampling due to abnormal uterine bleeding. Cells obtained from the cervix were distributed thinly and evenly on a glass slide and samples were promptly fixed by Carnoy's solution and normal spray fixatives.

In the present study, 40-60 year old women who had been married at least once and suffered from abnormal uterine bleeding, had no sexual intercourse within last 24 hours. They had not used vaginal shower or any lubricants within last 48 hours and vaginal cream within the last week before sampling.

Study participants signed a written informed consent. The sample collectors and a cytologist asked the participants to fill in a questionnaire including demographic information and specialized data related to women's diseases. Each patient was placed in the lithotomic position and a speculum was slowly inserted in their vaginas. Hot water was used to drive the speculum. The cervix and vagina were carefully examined. After observing uterine bleeding, two Pap smear samples were taken from each patient by the usual method. Then one of the samples was fixed by normal spray fixative and the other one was fixed by Carnoy's solution for 20 minutes. Samples were provided in a criss-cross manner in order to avoid bias. For the first patient, sample 1 and sample 2 were fixed by normal fixative spray and Carnoy's solution and for the next patient sample 1 and sample 2 were fixed by Carnoy's solution and normal fixative spray, respectively.

A pathologist determined the percentage of blood on each slide based on the number of red globules in each microscopic field of vision [13]. In the present study, the clarity of slides was categorized based on the percentage of slides covered with blood and the quality of staining the core and cytoplasm as follows: good (0-40%), average (40-70%) and bad (70-100%). The clarity of slides was determined in the following categories: good core staining and cytoplasm (proper staining), average (weak/ proper staining) and bad (weak core and cytoplasm staining) [12,13].

Then the samples were stained using Papanicolaou method and were studied by two cytologists using double-blind trial and results were recorded.

The data were statistically analyzed using SPSS software. Results from studying Carnoy's solution and spray-fixed slides in both methods were compared based on data sheets. This study used the statistical tests of thet-test, McNemar and Wilcoxon.

Results: In this study, out of 204 slides provided from cervical cells, 82 did not have the necessary cell adequacy. Demographic data showed that the mean age of clients, the mean age of the first marriage and the mean menarche age was 45.43, 17.39 and 13, respectively. The average duration of menstruation and average interval of menstruation was 10.14 and 28.68, respectively. 83.3% of women had irregular menstruation and 16.7% were menopause. The median of the number of pregnancies, delivery and abortion was 4, 4 and 0, respectively.

In the present study, cell adequacy of slides was investigated by the presence of squamous, columnar and transitional zone cells in Carnoy's-fixed and spray-fixed slides. Results showed that the presence of squamous cells in Carnoy's-fixed slides was more than spray-fixed slides and the difference was significant based on McNemar statistical test ($p=0.024$). This shows that Carnoy's solution had more power in cleaning blood from the slides but the presence of columnar cells and transitional zone in Carnoy's-fixed and spray-fixed slides was the same. The endometrial cells were also present.

The percentage of blood on slides in both methods was determined based on the number of red globules within the microscopic field of vision. The difference between the mean percentages of slides covered by blood in both methods was statistically significant (figures 1, 2). The mean number of slides covered by blood in spray-fixed was 67.99%, while it was reduced to 49.75%, respectively ($p=0.001$) in Carnoy's-fixed slides. The clarity of Carnoy's-fixed slides was more than spray-fixed slides ($p=0.001$) (figures 3,4). In this study, there was no significant difference between both methods in identifying inflammatory cells, pathogenic organisms, metaplastic and Keratinized cells. There were no abnormalities of epithelial and columnar cells in both methods.

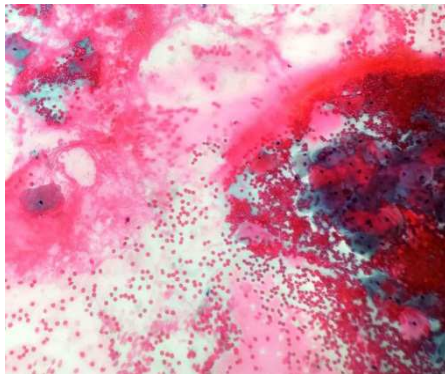


Figure 1: Spray-fixed smears

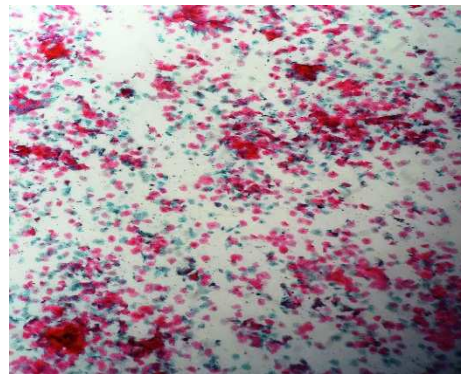


Figure 2: Carnoy's-fixed smears

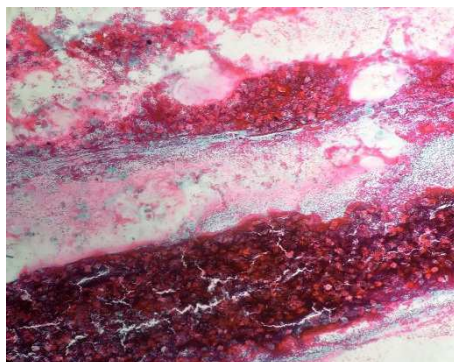


Figure 3: Spray-fixed smears

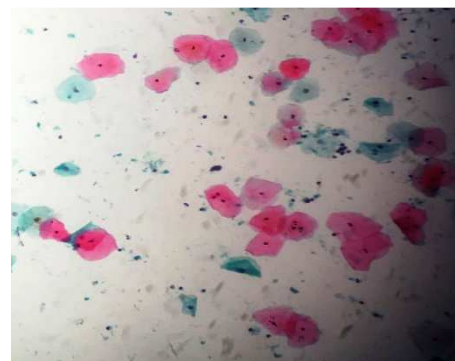


Figure 4: Carnoy's-fixed smears

Discussion: Bloody Pap smear has been one of the old problems of cytologists in studying samples. This has brought about changes in popular techniques of Pap smear. Using materials such as Saponin, thin prep and cytorich-red solution can reduce red globules from samples [14, 15]. It is obvious that using a solution which has the feature of cleaning the blood from surface of slides. Besides the fixation of cells has a great importance in the popular technology of Pap smear. Carnoy's solution is characterized by lysing red globules in different stages of fixation of pathology samples. This solution has been used in order to fix tissue samples of breast, thyroid, liver and womb. Carnoy's solution has also been used in cytology (Shamsi's study) to clean Pap smear bloody samples covered with blood due to contact bleeding (little blood). Can this solution clean the great amount of blood which exists in Pap smear samples of women with abnormal uterine bleeding? The present study investigated different aspects of this question.

The criteria for cell adequacy included the presence of squamous cells, columnar cells and cells from transitional zone. Shahidoleslam *et al*'s study (2004) used thin prep method in the U.S. They examined 57296 thin prep samples during 1 year. About 4767 samples were satisfactory before reprocessing (8.32%), while 2593 samples were covered with blood. After the repeated fixation by a thin prep solution to which acid citric was added, 1633 out of 2593 samples had reached cell adequacy level [16]. Results of the present study showed that cell adequacy and cell clarity

in Carnoy's-fixed slides was more than spray-fixed slides. The present study also indicated that Carnoy's solution remarkably causes a reduction in the number of red globules on the slide and an increase in its clarity.

In another study, Shamsi et al (2008) compared Carnoy's solution and ethyl alcohol 96% in fixation of Pap smear bloody samples that were covered with blood as a result of contact bleeding (little blood) in Shiraz. They aimed to study 410 out of 450 samples had the necessary cell adequacy. Results showed that the presence of squamous and columnar cells in Carnoy's-fixed slides was more than that of the alcohol-fixed ones. The reduction in the number of red globules on slides and the increase of clarity in Carnoy's-fixed slides was more than alcohol-fixed ones [17]. In Nicolasagoff et al's study (2002) that was done using thin prep method, 455 out of 583 unsatisfactory samples were repeatedly fixed using a thin prep solution to which acid acetic had been added and finally 257 samples became satisfactory or SBLB (satisfactory but limited) and 198 samples remained unsatisfactory [18].

The results from earlier and the present study show that cleaning blood from slides by solutions that lyse red globules may result in better identification of squamous, columnar and transitional zone cells and provide a more desirable cell adequacy. In this study, the difference between identification and distribution of inflammatory cells, microbial factors, metaplastic and keratinized cells in both methods was not significant. In the present study, the ability of Carnoy's solution in identification of cervical abnormalities was based on the Bethesda system. The findings were based on studying general classification of abnormalities and identification of abnormalities of squamous and glandular cells according to cytology form; no abnormalities were seen in epithelial and columnar cells in both methods. The presence of endometrial cells in Carnoy's-fixed and spray-fixed slides was the same.

Table1. Comparing the mean percentage of bloody smears in spray-fixed and Carnoy's-fixed slides

Method Criteria	Spray		Carnoy's	
	Mean	SD	Mean	SD
Percentage of Bloody Slides	67.99	29.25	49.75	33.51
P-value	0.001			

Table2. Comparing the median and quartile range of the clarity of slides in spray-fixed and Carnoy's-fixed slides

Method Criteria	Spray		Carnoy's	
	Median	Quartile Range	Median	Quartile Range
Slide's Clarity	3	(2-3)	2	(1-3)
P-value	0.001			

*1: Good Clarity 2: Average Clarity 3: Bad Clarity

The study results revealed that Carnoy's solution had a positive effect on the fixation of Pap smear bloody slides that were greatly covered by blood. This solution is also effective on lysing red globules from slides and may increase the clarity, provide a better identification of squamous cells and consequently increase the cell adequacy. As a popular method, Carnoy's solution is an effective fixative in Pap smear bloody slides and has no effects on the identification of endometrial cells and their abnormalities. The results of this study can be noticed by healthcare and treatment administrators to provide Pap smear samples in the first reference of patients even in case of bleeding, and consequently reduces specialists' and midwives' anxiety about low quality of slides that makes patients not to show off again for providing Pap smear and continuing the treatment process.

It is suggested to repeat this study using different percentages of acid acetic in Carnoy's solution (preparing different kinds of Carnoy's solution) and to compare them with each other with a bigger sample size.

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