Asian Journal of Medical Sciences 5(2): 41-43, 2013
ISSN: 2040-8765; e-ISSN: 2040-8773

Submitted: September 22, 2012 Accepted: November 29, 2012 Published: April 25, 2013

Comparative Studies of Dry and Wet Cervical Smear in Human

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Abstract: Cervical cancer is on the increase in women all over the world prompt and regular screening especially in rural areas has always been challenging. This study was carried out to compare a dry slide cervical smear with the conventional wet slide cervical smear in order to adopt an alternative. Six months samples of cervical smears were collected. 100 paired cervical smears slides were treated. One as conventional wet fixed and the other as fixed dried and rehydrated prior to staining. The nuclear, cytoplasm and background were compared. The nuclear of air dry was satisfactory compared to wet (9.0% versus 10.0%) also cytoplasm (8.4% versus 8.6%) the background of air dried rehydrated smear were clearer. This could be as a result of dehaemoglobinization of red blood cells, rendering it transparent. From the result it is possible to use air dried rehydrated cervical slide for cervical smear screening.

Keywords: Air dried smear, cervical, fixed pap smear, red blood cell, rehydrated, wet smear

INTRODUCTION

PAP smear as a useful method for early detection of cancerous lesions and inflammatory conditions is routinely carried out worldwide. Smears are collected by gynecologists or paramedical staff in clinics, hospitals or health centrist.

A number of fixatives are used in exfoliative cytology, the common ones are modifications of 95% ethylalcohol. This can be used on its own with satisfactory results, but the addition of 3% glacial ascetic acid increase the nucleo-protein-fixing properties (Bancroft and Stevens, 1982). This is the standard fixative in most laboratories and gives excellent nuclear and cytoplasmic morphology. Where slides have to be send by post, wet fixatives are prohibited by status so aerosol spray fixatives containing polyethylene glycol in ethyl alcohol were introduced (Bancroft and Stevens, 1982).

The present study aimed to investigate comparative studies of dry and wet cervical smear in human.

METHODOLOGY

This study was carried out in July, 2011 in Ahmed Bello University Teaching Hospital Zaria. One hundred (100) cervical smears were collected over a period of three month from patients who attended Ahmed Bello University Teaching Hospital Zaria for cervical cancer screening. The age of the women ranged from 21-65 years, menstruating and pregnant women were excluded from the study. The smears were collected by gynecologist by means of wooden Ayre spatulas. One set of slides was immediately fixed in 95% ethanol for 30 min and labeled Wet Fixed (WF) the other set was air-dried for 2 hours rehydrated prior to staining labeled Dried Fixed (DF). Then the slides were pooled and stained by standard papanicolaus method and examined by a cytopathologist.

RESULTS

This study revealed that immersion of air dried PAP smears in water for 30 sec lysed red blood cells effectively but retained squamous and glandular cells present (Fig. 1). In fact, the clearer background attributed to the lysis of red blood cells in dried fixed smears facilitates the interpretation of smears and the quality of staining is preserved equal or superior though, not statistically significant. The procedure of air-dried and rehydration is simple, fast, inexpensive and highly effective for the lysis of red blood cells, as evidenced by a pinkish color in water after rehydration time is 30 sec (Fig. 1 and 2), because this found to be the optimal time for cleaning up the background this renders search for diagnostic cells less tedious and avoids the problems of overlapping red blood cells obscuring cellular details.

DISCUSSION

Pap is the accurate stain for assessment of chromatin in cervical smears and ensures optimal resemblance to corresponding cells in histologic sections; however, a subject is usually immediate fixation in 95% ethanol (Chan and Kung, 1988). The rate of air-drying can not be prevented around the edges of smears where diagnostic cells are populated. Effects

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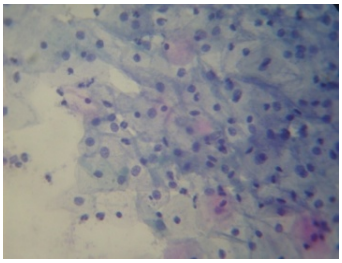

Fig. 1: Photomicrograph of a section of cervical smear prefixed and air dried (DF). Note the absence of RBC's (Red Blood Cells), Clear background and well outlined nuclear appearance (N). Papanicolaou Stain X 400

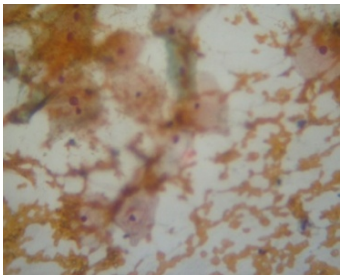

Fig. 2: Photomicrograph of a section showing same sample of cervical smear as in Fig. 1 but fixed normally in 95% ethylalcohol (WF). Note the preserved RBC's (R) obscuring the Epithelial Cells (EC). Papanicolaou Stain X400

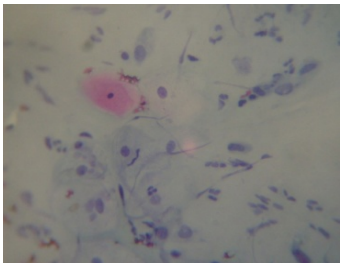

Fig. 3: Photomicrograph showing a section of prefixed, air dried cervical smeared sample. Note the clear background, few RBC's and well outlined epithelial cells with well outlined nuclei. Papanicolaou stain X 400

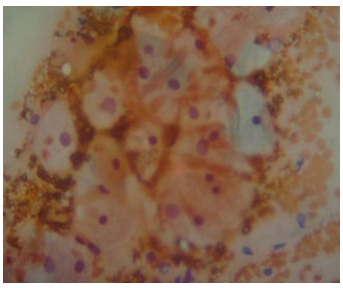

Fig. 4: Photomicrograph of the same section of cervical smear as in Fig. 3 showing large amount of RBC's obscuring view. Papanicolaou stain X 400

of air-drying on nuclear and cytoplasmic staining are visiable if the amount of aspirate is small and relatively dry. For this popurse it is necessary to rehydrate these dried up cells. This study investigated that, immersion of air-dried pap smears in water for 30 seconds lysed red blood cells effectively but retained squamous and glandular cells present. The results of this study is similar to previous study of rehydration of cervical smears with regard to red blood cell lysis but different from them with respect to cytoplasmic staining since that study revealed superiority in cytoplasmic staining of Air-Dried Rehydrated Fixation (ARF) smears (Sivarman and Iyengar, 2002: Gupta *et al*., 2003). The procedure of air-drying and rehydration is simple, fast, inexpensive and highly effective for the lysis of red blood cells, as evidenced by a pinkish color in normal saline after rehydration of heavily blood stained smears. The rehydration time is 30 sec because this was found to be the optimal time for clearing up the background (Ng *et al*., 1994). This renders search for diagnostic cells less tedious and avoids the problem of overlapping red blood cells obscuring cellular details. Longer immersion in normal saline is detrimental because of nuclear wrinkling. The proper rehydration fluid is normal saline as other rehydration fluids (hypotonic solutions, tap water or aqueous glycerin) were proved to lyse nucleated cells as well as red blood cells (Ng *et al*., 1994). Rehydration is recommended for several other advantages as well. In this study we use water as the rehydrated fuid; however the result is as similar as that of the normal saline. The smears can be spread more thinly and leisurely, the problem of falling off larger particles or thicker portions of the smear in wet fixation can be avoided, the cells have better adhesion to slides, the cells are flatter and the depth of focus on nuclei is much shallower, which is a great advantage in taking photomicrographs (Chan and Kung, 1988). This procedure can also be used for Fine Needle Aspiration Biopsy (FNAB), effusion cytology and exfoliated cells (Chan and Kung, 1988, Shidham *et al*., 2001, Jones, 1996) and for other staining methods such as H and E (Lencioni *et al*., 1954), immunohistochemical staining (Shidham *et al*., 2000; 2003) and Giemsa (Schulte and Wittekind, 1987).

CONCLUSION

Rehydration of air-dried cervical smears (Fig. 3) is a suitable alternative to wet-fixed smears (Fig. 4). The staining quality is either the same as or better than wet-fixed smears and the unsatisfactory rate is lower. This

technique is simple and can be conveniently adopted in resource limited settings.

ACKNOWLEDGMENT

We wish to acknowledge the technical assistance of the laboratory staff of the Department of Histopathology, Faculty of Medicine Ahmadu Bello University, Zaria, Nigeria.

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