

REHYDRATION OF AIR-DRIED CERVICAL SMEARS: AN ALTERNATIVE TO ROUTINE WET FIXATION

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Abstract- To prepare Pap smears, the routine practice is to fix the slides immediately in 95% ethanol. This study was performed to evaluate the possibility of routine use of alternative method of air-drying and rehydration prior to alcohol fixation instead of conventional method. Paired cervical smears with at least low cellularity were collected from 117 women who participated in the study. One set was labeled WF (wet fixed or fixed immediately in 95% ethanol) and the other one ARF (air-dried, rehydrated and fixed). The latter further split into 3 subgroups based on the duration of air-drying. The staining quality of the slides was assessed with respect to chromatin, nuclear and cytoplasmic borders, cytolysis, cellularity, cytoplasmic staining, and red blood cells lysis. Then they were graded blindly. The results were analyzed by Chi square test to compare the defined parameters between the 2 groups and also the 3 subgroups. ARF slides were significantly better with regard to clearance of background due to the lysis of red blood cells (P value, 0.000, x2 test; kappa, -0.27). No statistically significant differences were noted between two groups in terms of other cytologic features. Cytologic features of ARF slides were statistically identical to WF smears. However, red blood cells lysis rendered clearer background in ARF slides. Air-drying and rehydration of slides is a superior method for heavily blood stained smears and can be used at least with identical quality for routine practice.

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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 centers. The routine practice is to fix the slides immediately in 95% ethanol and send them to laboratory to be stained and evaluated by cytopathologist.

Unfortunately, improper fixation and drying artifacts are common due to inadequate training of workers or heavy workload (1-2). That leads to repeating smears, and increasing the workload or missing the patients.

One alternative method for overcoming the problem of poor fixation, introduced over half a century ago, is air-drying followed by rehydration and fixation before staining in laboratory (3). This method has been offered for fine needle aspiration (FNA), effusion cytology and exfoliated cells, but few reports regarding it as a substitute technique for cervical smears are available (4-8).

The current study was undertaken to evaluate the possibility of the routine use of rehydration of air-dried Pap smears before fixation in ethanol.

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MATERIALS AND METHODS

Over a period of six months, 117 cellular paired cervical smears were obtained from women who attended Akbarabadi Hospital, a specialized center for gynecology and obstetrics, for routine check-ups or clinical indications. We obtained informed consent from all participants.

The age of the patients ranged from 17 to 75 years. Menstruating or pregnant women were not included in the study. The smears were taken by a gynecologist under direct vision using wooden spatulas. One set of slides was immediately wet fixed in 95% ethanol and labeled WF. The other set was air-dried, rehydrated, and fixed (ARF). The latter group was allowed to air-dry by placing the slides on a rack exposed to room air for 3 different periods of time (2, 24 and 48 hrs). After that they were rehydrated by being immersed in normal saline for 30 seconds followed by fixation in 95% ethanol. Then the slides were coded, pooled and stained by standard Papanicolaou method and examined blindly by a cytopathologist.

The new Bethesda system of reporting cervicovaginal smears (2001) was adopted for diagnosis, and the staining quality of smears was determined by various parameters as follows: cellularity (high/ intermediate/ low), cytolysis (0+/ 1+/ 2+/ 3+), cell borders (distinct/ indistinct), cytoplasmic staining (excellent/ satisfactory/ unsatisfactory), nuclear borders (distinct/ indistinct), chromatin (hazy/crisp). The above criteria were assessed in both squamous and glandular cells and a score was assigned to each slide to judge the cellular preservation and staining quality. The presence or absence of red blood cells and infections was also noted. The smears were decoded and separated into WF and ARF groups. The latter was further split into 3 sets with respect to duration of air drying to determine the effect of time on the quality of staining. Slides with scanty or no cellularity did not enter the study from the beginning, so all the 117 paired slides were used for statistical analysis.

Using SPSS software, version 13, a comparison of 2 major groups and 3 subgroups was made using the χ^2 test. P value less than 0.05 was considered significant.

RESULTS

Among 117 smears reported by the new Bethesda system, none of the slides in both groups was unsatisfactory due to poor fixation.

Excellent cytoplasmic staining of squamous and glandular cells was seen in 44 (37.6%) smears in the ARF group, while 40 (34.2%) smears in the WF group revealed the same. Similarly, cytoplasmic staining in the ARF group was satisfactory in 77 (65.8%) smears as compared to 73 (62.4%) in the WF group. Sixty six (56.4%) of WF slides and 69 (58.9%) of ARF ones had high cellularity. Eighty five (72.6%) of WF slides and 94 (80.3%) ARF ones disclosed no cytolysis (Table 1). The above results were better in the ARF group but proved to be statistically comparable in cellularity, cytoplasmic staining, and cytolysis ($P < 0.001$, χ^2 test). The number of slides with no red blood cells was 83 (70.9%) and 110 (94%) for WF and ARF ones respectively. Slides with 1+ red blood cells were seen in 15 (12.8%) of WF smears and 4 (0.03%) of ARF ones. 12 (10.2%) of WF slides and 3 (0.02%) of ARF ones revealed +2 red blood cells. Four (0.03%) and 3 (0.02%) of WF slides had 3+ and 4+ red blood cells respectively, but none of the ARF slides exhibited 3+ or 4+ red blood cells (Figure 1). These differences in red blood cells were statistically significant (P value = .000; χ^2 test, $\kappa = -0.27$). 115 (98.2%) smears of both groups revealed distinct cell borders. Nuclear border was distinct and chromatin pattern was crisp in all smears of two groups. As a whole, statistically significant similarities were seen between the two groups with regard to cellularity, cytoplasmic staining, nuclear chromatin, amount of cytolysis, and distinction of nuclear and cell borders. When the two groups were compared in terms of cytologic diagnosis, the results were in agreement. All specific infections as well as epithelial cell

Table 1. Degree of cytolysis in ARF and WF smears. ARF smears show less cytolysis, though the difference is not statistically significant.

Cytolysis	0	1+	2+	3+
ARF smears	94	16	6	1
WF smears	85	26	5	1

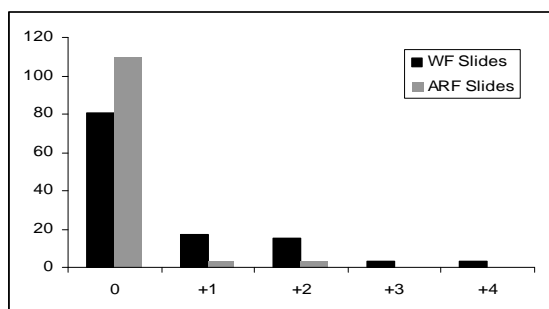


Fig. 1. Degree of red blood cells in WF and ARF slides. ARF smears have lower number of red blood cells.

abnormalities could easily be picked up in the ARF group. Regarding cellularity, cytoplasmic staining and cytolysis, comparison of various periods of air-drying revealed better results in slides rehydrated for 2 hours than 24 and 48 hours, and the results were statistically comparable. Cellular and nuclear borders and chromatin pattern were similar in the 3 subgroups. The clearance of background was statistically better in slides air-dried for 24 hours in comparison with paired WF smears ($P < 0.01$; χ^2 test, $\kappa = -0.31$; Figures 2-5). In the other two groups, red blood cell lysis was better than paired WF smears, but the difference was not statistically significant.

DISCUSSION

Pap is regarded as the best stain for assessment of chromatin pattern in cervical smears and ensures maximum resemblance to corresponding cells in histologic sections; however, a prerequisite is usually immediate fixation in 95% ethanol (4). Some degree of air-drying is inevitable particularly at the edges of smears where diagnostic cells often concentrate. Deleterious effects of air-drying on nuclear and cytoplasmic staining are particularly noticeable if the amount of aspirate is small and relatively dry. So, it is highly desirable to use methods to rehydrate these dried up cells.

This study revealed that immersion of air-dried pap smears in normal saline for 30 seconds lysed red blood cells effectively but retained squamous and glandular cells present. In fact, the clearer background attributed to the lysis of red blood cells in ARF smears facilitates the interpretation of smears and the quality of staining is preserved equal or superior though not statistically significant. The obtained results were similar to those of previous

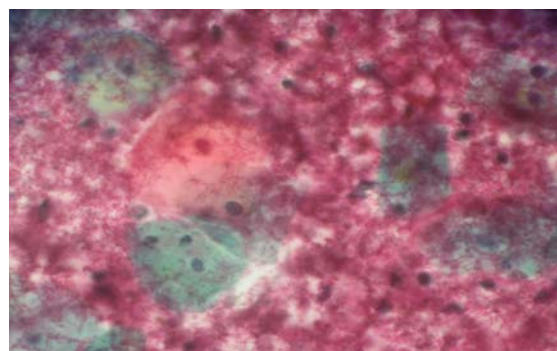


Fig. 1. A wet fixed Pap smear slide. The background is not clear due to excess blood ($\times 100$).

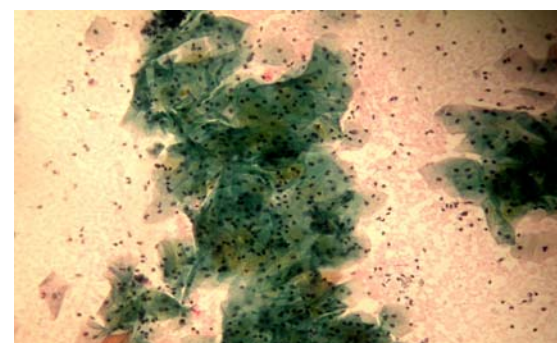


Fig. 2. The paired ARF slide of the above Pap smear. The background is clearer. The cytologic staining quality is excellent ($\times 100$).

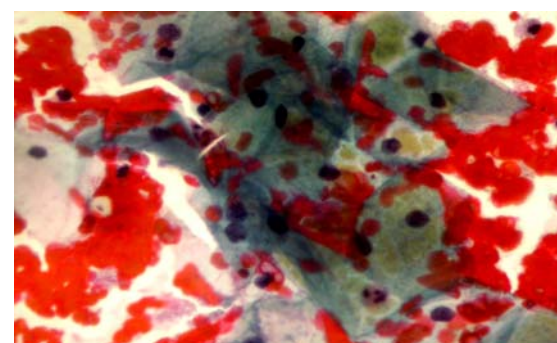


Fig. 3. The slide in fig 1 shown by higher power ($\times 400$).

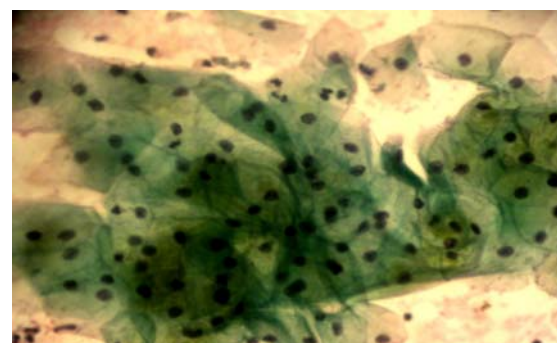


Fig. 4. The slide in fig 2 shown by higher power ($\times 400$).

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 ARF smears (1); nevertheless, the results of this study were similar to another study in both respects (2). 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 seconds because this was found to be the optimal time for clearing up the background (5). 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 best offered 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 (5). The maximum time of air-drying with well preservation of cellular details was up to four days (1). Rehydration is recommended for several other advantages as well: 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 (4). This procedure is also used for FNA, effusion cytology and exfoliated cells (4-8), and for other staining methods such as H&E (3), Giemsa (9) and immunohistochemical staining (10-11). According to the current study, we recommend air-drying for 24 hours and rehydration prior to fixation for blood-stained pap smears in addition to traditional wet fixation or as a substitute for it. Though there was no difficulty in reaching cytologic diagnosis with rehydrated smears, and in fact the results were identical or superior to wet fixed smears, for non-blood stained smears its role is less clear. Nonetheless, the routine use of the method might result in better smears.

Conflict of interests

We have no conflict of interests.

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