RESEARCH COMMUNICATION

Anal Cancer Screening by Modified Liquid-Based Cytology in an HIV Clinic

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Abstract

This study aimed to screen for anal cancer and to determine its cytomorphology using liquid-based cytology (LBC) with specimens preserved in 95% ethyl alcohol. Anal swabs were collected for cytological examination from 177 adult, HIV-infected patients. After collection, sample slides were reviewed and classified according to their cytomorphology using the modified Bethesda 2001 system. An abnormal anal Pap smear was found in 26.0% of the patients. The diagnoses were: 66.7% negative for intraepithelial lesions (NIL), 14.1% with atypical squamous cells of undetermined significance (ASC-US), 10.7% (19) with low-grade squamous intraepithelial lesions (LSIL), and 1.13% with high-grade squamous intraepithelial lesions (HSIL). The cytological evaluation was an unsatisfactory result only with 6.67%. The present modified LBC using 95% ethyl alcohol as the preservative could thus be used for anal cancer screening. The number of SILs in Thai HIV-infected patients is lower than that in Western countries. We found anal cytology a satisfactory tool for early screening and detection of anal dysplasia commonly found in high-risk, HIV-infected patients.

Keywords: Anal carcinoma - HIV - screening - cytology

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Introduction

Implementation of the Pap smear in women was responsible for reducing the incidence of cervical cancer between 1955 and the mid-1980s (National Cancer Institute, 2011). Interestingly, anal carcinoma has many similar histopathological characteristics in common with cervical cancer thought to arise from squamous intraepithelial lesions in the anal canal. Anal cancer is uncommon in the general population, with incidence rates of approximately 2 cases per 100,000 population in the United States. Notwithstanding, persons infected with human immunodeficiency virus (HIV) have a higher risk for anal cancer. Consequently, similar screening of populations at high risk of anal squamous intraepithelial lesions (ASILs), it is hoped, would reduce the incidence of anal cancer.

Anal smears are increasingly being used as a screening test for anal squamous intraepithelial lesions (ASILs). In certain high-risk populations, such as men who have sex with men (MSM) and human immunodeficiency virus (HIV)-positive men and women, the incidence of anal cancer has been estimated 35 cases per 100,000 population. In HIV-positive MSM, the risk of anal cancer has been estimated at more than two-fold that of non-HIV-infected persons (Johnson et al., 2004; Siegel et al., 2012; Silverberg et al., 2012).

Liquid-based cytology (LBC) has become a common screening method for detecting precancerous lesions as well as diagnosing cervical cancer. In a pilot study, we found by using the modified LBC method, cell preservation was better and there were not any problems with air-drying artifact of the anal smear; that is, compared to the conventional Pap smear protocol. In the current study, anal cancer was screened using the modified LBC (with 95% ethyl alcohol used as the preservative) and cytomorphological assessment of the anal smears performed to screen for anal cancer.

Materials and Methods

Study Population

The study protocol was approved by the Ethics Committee of Khon Kaen University. Anorectal swabs for cytological examination were collected (between June 2010 and February 2011) in liquid medium from 177 adult, HIV-infected patients at Srinagarind Hospital. The exclusion criteria were: (a) presence of gastrointestinal hemorrhage (b) intestinal obstruction and (c) anal wound. The samples were collected from the anal canal using a Rayon swab. The anal swab was then placed in 95% ethyl alcohol liquid-based solution for preservation.

Processing of modified LBC

The specimens were collected in centrifuge tubes containing 5 ml of 95% ethyl alcohol. The supernatant

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was discarded using an auto-pipette, then a drop of sample (200 µl) placed onto Superfrost plus slides (2 areas). Each droplet was smeared to 2 cm in diameter. The smear was allowed to air-dry at room temperature before it was fixed onto the slide in 95% ethanol until being stained by Papanicolaou’s technique (Laiwepithaya et al., 2008). The samples were classified according to the modified Bethesda 2001 System Terminology recommended for cervical smears (Solomon et al., 2002).

The diagnoses included: (a) Unsatisfactory because the number of cells was <6 nucleated squamous cells per 40x high power field (nsc/hpf) (Arafin et al., 2005); (b) Negative for intraepithelial lesion or malignancy (NIL), (c) Atypical squamous cells of undetermined significance (ASC-US), (d) Atypical squamous cells of undetermined significance which could not exclude high grade squamous intraepithelial lesion (ASC-H), (e) low grade squamous intraepithelial lesion (LSIL), and (f) high grade squamous intraepithelial lesion (HSIL). The slides were reviewed by two cytopathologists and evaluated for cellularity and presence of anucleated squamous cells, glandular/squamous metaplastic cells, parakeratotic cells, perinuclear halo-cells, bi-/multi-nucleated squamous cells, koilocytes and dysparakeratosis. The number of cells exhibiting each of these morphologic features was recorded as ‘none’ or ‘present’. The cellularity was checked by one pathologist.

Analysis
The demographic characteristics were summarized for the study population using frequencies and percentages for the categorical variables and median for the continuous variables. Analyses were done with MedCalc Software Version 12.3.0 (Broekstraat 52, 9030 Mariakerke, Belgium). The odd ratios and 95% confidence intervals (CI) for establishing significant differences was set at p<0.05 (MedCalc, 2012).

Results
The median age of the HIV-infected patients was 39.4 years (range, 17-67). Among the 177 patients 120 were males. A satisfactory specimen required that the number of cells be more than 6 nsc/hpf (Figure 2A). An abnormal anal Pap smear occurred in 26.0% in these patients. The diagnoses included: 6.67% (12) unsatisfactory; 67.23% (119) NIL; 14.12% (25) ASC-US; while 10.74% (19) were LSIL with koilocytes (or HPV changed in 16 of them) (Figure 2A) and 1.13% (2) were HSIL (Figure 2B).

Retrieved slides were reviewed for cellularity and the presence of anucleated squamous cells, glandular/squamous metaplastic cells, parakeratotic cells, perinuclear halo-cells, bi-/multi-nucleated squamous cells, koilocytes and dysparakeratosis. Anucleated squamous cells, present in 99.15% (118/119) of smears, were NIL, while 96% (24/25) were ASCUS and 100% (21/21) were SIL. Glandular/squamous metaplastic cells (Figure 2B)

Table 1. Relative Satisfactoriness of Modified LBC with Cytomorphology

| Cytomorphology          | NIL    | ASCUS  | SIL    | Odds ratio: (95% CI) | p value | Odds ratio: SIL (95% CI) | p value |
|-------------------------|--------|--------|--------|----------------------|---------|-------------------------|---------|
| Anucleated cells        | 118    | 24     | 21     | 0.20 (0.01-3.36)     | 0.266   | 0.54 (0.02-13.81)       | 0.712   |
| Glandular/Squamous cell | 72     | 25     | 15     | 0.65 (0.34-1.27)     | 0.208   | 1.63 (0.59-4.50)        | 0.344   |
| Parakeratotic cells     | 23     | 12     | 11     | 3.85 (1.55-9.54)     | 0.004   | 4.59 (1.74-12.11)       | 0.002   |
| Perinuclear halo-cells  | 7      | 8      | 6      | 7.52 (2.42-23.44)    | 0.001   | 6.4 (1.89-21.59)        | 0.002   |
| bi-/multi-nucleated     | 12     | 8      | 16     | 4.19 (1.49-11.76)    | 0.006   | 3.57 (1.16-10.92)       | 0.025   |
| Koilocytes              | -      | -      | 16     | -                    | -       | 717.0 (37.88-13569.6)    | 0.0001  |
| Dysparakeratosis        | -      | -      | 11     | -                    | -       | 261.76 (14.39-4761.6)    | 0.0002  |

![Figure 1. Modified LBC Pap Smear Shows: A) Adequate Squamous Epithelial Cells; and B) Glandular Epithelial Cells. Papanicolaou stain (x400).](image)

![Figure 2. Representative Examples of Modified LBC Pap Smear Showing: A) LSIL with HPV Infection with Koilocytes; and B) HSIL (Arrow). Papanicolaou stain (x400).](image)
were present in 60.5% (72/119) of NIL, 100% (25/25) of ASCUS and 71.42% (15/21) of SIL. The smears in ASCUS and SIL were observed to have 48% (12/25) and 58.38% (11/21) parakeratotic cells, which was often greater than 3-fold the NILs (19.32%) (p<0.05). The research found that ASCUSs and SILs were characterized by perinuclear halo-cells in 32% and 28.57%, which was often greater than 6-fold the NILs (5.88%) (p<0.05). Bi-/multi-nucleated ASCUSs, and SILs were characterized in 32% and 76.19% which were often greater than 3-fold the NILs (10.08%) (p<0.05). The SIL had koilocytes and dysparakeratosis demonstrating the statistically significant presence of NIL (p<0.05) (Table 1).

Discussion

In the current study, we analyzed anal cytology (Pap testing). Anal Pap screening is very simple, painless and quick. ThinPrep and conventional smears of the anal canal yielded similar diagnostic results but the ThinPrep technique reduces fecal and bacterial contamination and the air-drying artifact, which frequently hinders cytologic evaluation of the anal canal. In the current study, 95% ethyl alcohol solution was successfully used as a good preservative; resulting in a modified liquid-based cytology for screening anal cancer. The smear reduces the effective cost and reduces air-drying artifact. The number of ASILs among Thai HIV-infected patients is lower than found in patients in Western countries (Darragh et al., 1997).

We found the frequency of an abnormal anal Pap smear was about 26% (including ASC-US, LSIL and HSIL): cytological abnormalities in HPV-positive MSM in the USA and Europe have varied between 27% and 81% (Palefsky et al., 2005; Chiao et al., 2006). In the current study, 95% ethyl alcohol solution was successfully used as a good preservative; resulting in a modified liquid-based cytology for screening anal cancer. The smear reduces the effective cost and reduces air-drying artifact. The number of ASILs among Thai HIV-infected patients is lower than found in patients in Western countries (Darragh et al., 1997). We found the frequency of an abnormal anal Pap smear was about 26% (including ASC-US, LSIL and HSIL): cytological abnormalities in HPV-positive MSM in the USA and Europe have varied between 27% and 81% (Palefsky et al., 2005; Chiao et al., 2006). HIV-infected patients engage in high-risk behavior which exposes them to HPV. The tested screening method can contribute to the prevention of anal cancer and should be evaluated further. In this study, however, almost all of the ASIL was asymptomatic and all HIV-infected patients were offered anal Pap smears but they did not undertake HPV testing. Future studies need to confirm HPV testing and HPV typing.

The role of anal Pap smear screening in the Thai setting has not previously been examined. Elsewhere, however, anal cytology has been shown to be a cost-effective screening method for detection of ASIL in populations at high risk of anal carcinoma (Goldie et al., 1999). Screening positive for anal cancer allows patients to be referred for anoscopy and biopsy. The early detection of anal cancer could potentially improve survival. Anal SCC, when detected as a localized disease, has a 78% 5-year survival rate compared with 18% for metastatic disease (Johnson et al., 2004).

Among the smears studied in the current research, the number of anucleated squamous cells and glandular/ squamous metaplastic cells correlated well with the cytological diagnosis. Almost all of the anal specimens presented anucleated squamous cells which may have obscured abnormal cells. The presence of glandular/ squamous metaplastic cells was a prerequisite for indicating collection adequacy. (That is, specimens were deep in the transformation zone, which is the most common area for anal cancer to occur.) Smears with glandular/squamous metaplastic cells comprised 67.9% (112/165): of these, 60.50% (72/119) were NIL, 100% (25/25) were ASCUS and 71.42% (15/21) were SIL. The presence of glandular/squamous metaplastic cells correlated statistically with the chance of detecting abnormal lesions. We did not encounter any cases of atypical glandular cells of undetermined significance, glandular dysplasia, or adenocarcinoma in our smears.

Parakeratotic cells, although frequently observed, were not helpful in the diagnosis of ASIL (Friedlander et al., 2004). The ASCUS and SIL had parakeratotic cells, perinuclear halo-cells and bi-/multi-nucleated; demonstrating a statistically significant >3-fold predominance of NIL, (p<0.05) (Table 1). Parakeratotic cells, perinuclear halo-cells, bi-/multi-nucleated may be helpful indicators of abnormal cells. The SIL had koilocytes and dysparakeratosis, demonstrating a statistically significant presence of NIL salient feature of dysplastic cells. Koilocytes were the most reliable indicators of HPV infection, observed in 76.19% (16/21) of LSIL and 50% (1/2) HSIL. The koilocytes and dysparakeratosis in Pap smears were a reliable marker for anal intraepithelial neoplasia (AIN).

In conclusion, modified liquid-based cytology (with 95% ethyl alcohol solution as the preservative) can be used for anal cancer screening. Parakeratotic cells, perinuclear halo-cell and bi-/multi-nucleated are abnormal cells and clear indicators of abnormal cells. The number of SILs in Thai, HIV-infected patients is lower than that of similar patients in Western countries. We found anal cytology to be a useful tool for early detection of anal dysplasia among high-risk patients in the HIV-infected population.

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