Anal Cancer Screening by Cytology and HPV Testing in HIV-infected Patients Using Modified-LBC as Fixative

การตรวจคัดกรองมะเร็งทวารหนักทางเซลล์วิทยาและการตรวจหาเชื้อ HPV ในผู้ป่วยติดเชื้อ HIV โดยการใช้ modified-LBC เป็นน้ำยารักษาสภาพเซลล์

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ABSTRACT

The aim of this study was to determine cytology and HPV DNA testing for screening anal cancer using modified-liquid based cytology (modified-LBC) as fixative. Anal samples were collected from 166 HIV-infected patients to analyze by cytological exam and HPV DNA testing. We found the negative HPV in normal, ASCUS, LSIL and HSIL were 68.6%, 59.2%, 31.6%, and 0%, respectively. The positive HPV in normal, ASCUS, LSIL and HSIL were 31.4%, 40.8%, 68.4%, and 100%, respectively, that significant associated between the presence of abnormal cytology and HPV positive (p<0.05). The collected sample in the modified-LBC are effective to be used for screening anal cancer by cytology and HPV testing.

บทคัดย่อ

วัตถุประสงค์ในการศึกษานี้เพื่อนำ modified-LBC มาใช้เป็นน้ำยารักษาสภาพเซลล์ ในการตรวจคัดกรองมะเร็งทวารหนักทางเซลล์วิทยาและการตรวจหาเชื้อ HPV จากตัวอย่างเซลล์เยื่อบุทวารหนักของผู้ป่วยที่ติดเชื้อ HIV 166 รายนำมาตรวจทางเซลล์วิทยาและตรวจหาเชื้อ HPV พบว่าผลการตรวจ HPV ที่ให้ผลลบมีผลทางเซลล์วิทยาเป็น normal 68.6%, ASCUS 59.2%, LSIL 31.6% และ HSIL 0% ส่วนผลการตรวจ HPV ที่ให้ผลบวกมีผลทางเซลล์วิทยาเป็น normal 31.4%, ASCUS 40.8%, LSIL 68.4% และ HSIL 100% ซึ่งมีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ (p<0.05) วิธีการเก็บตัวอย่างด้วย modified-LBC มีประสิทธิภาพที่สามารถนำมาใช้ในการตรวจทางเซลล์วิทยาและการตรวจหาเชื้อ HPV เพื่อใช้ในคัดกรองมะเร็งทวารหนักได้

Keywords: Anal cytology, HPV, Fixative
คำสำคัญ: การตรวจทางเซลล์วิทยาของทวารหนัก การตรวจหาเชื้อ HPV น้ำยารักษาสภาพเซลล์

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Introduction

The anal cancer has striking similarity to cervical cancer both of etiology and pathogenesis. Both are associated with persistent infection of human papillomavirus (HPV) (Nyitray et al., 2013). The prevalence of HPV infection in anal cancer is detected in approximately 85-97% (Dupin et al., 2015). The incidence rate of anal cancer has increased in the last 25 years, particularly in at-risk population including HIV-infected individuals, which is about 30-fold higher than in general population (Weis et al., 2011). The weakening immune system of HIV-infected individuals is an additional risk factor of HPV infection that leads to anal intraepithelial neoplasia (AIN) and anal cancer (Van der Zee et al., 2013). The screening for cervical cancer by cytology is widely accepted as the most strategy to reduce incidence and mortality rate of cervical cancer, that the anal cytology can do the same results for anal cancer (Darragh, 2004). The anal cytology screening is recommended for all at-risk populations such as MSM, HIV-infected individuals, women with abnormal cervical cytology results and patient with organs transplant (Schim van der Loeff et al., 2014). In previously reported the anal cytology for detecting anal precancerous lesions is a moderate both of sensitivity (60-70%) and specificity (32-59%), the other test needed for improvement (Chebib, Duggan, 2014; Walts et al., 2014). HPV DNA testing is considered to as an adjunct test to cytology or either alone for anal cancer screening, due to HPV DNA testing has a greater sensitivity (75-100%) than cytology (Darragh, Winkler, 2011). The cells collection in liquid-based cytology (LBC) of the same specimen for cytology and HPV testing is becoming increasingly used across laboratory. Due to the fact that, modified-LBC using 95% alcohol as a preservative can be used for anal cancer screening (Patarapadungkit et al., 2012).

Objective of the study

The aim of this study was to determine cytology and HPV DNA testing in anal specimens using modified-liquid based cytology (modified-LBC) as fixative.

Methodology

Sample collection and preparation

This study was a cross-sectional study analysis of 166 HIV-infected individuals at Srinagarind Hospital. The study period included all patients who underwent anal cancer screening between June 2010 and February 2011. Besides, the exclusion criteria were; present of gastrointestinal hemorrhage, intestinal obstruction and anal wound. Anal specimens were collected with Rayon swab by gently inserted approximately 5-6 cm into the anal canal while rotating the swab to all side of anal canal (Darragh, Winkler, 2011). The anal swab quickly placed in modified-liquid based cytology using 95% ethyl alcohol as a fixative.

Modified-liquid based cytology

The anal cytology specimens were centrifuged at 1500 rpm for 10 minutes at room temperature, the sediment approximately 200 µl was transferred to positively charged slides. The slides were air-dried at room temperature after that fixed in 95% alcohol for at least 15 min, and Papanicolaou stains were performed to assess cellularity and morphology. All slides were evaluated independently by 2 cytologists. The cytology results were categorized following to the Bethesda system 2001 as

normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells cannot rule out high-grade squamous intraepithelial lesion (ASC-H), High-grade squamous intraepithelial lesion (HSIL), and squamous cells carcinoma (SCC) (Darragh, Palefsky, 2015).

HPV DNA testing

Anal cells were washed with 1x phosphate buffered saline by centrifugation at 13,500 rpm, 4°C for 5 minutes and DNA was extracted from anal specimen using the AllPrep DNA mini kit (Qiagen, Valencia, USA) according to the manufacturer instruction. As a quality control measure, DNA was evaluated by amplification of the β-globin gene as an internal control for DNA adequacy.

Qualified samples from positive β-globin gene were subjected to HPV DNA detection by real-time PCR on a LightCycler 480 instrument (Roche Diagnostic). A 142-bp fragment from L1 region of HPV DNA was amplified using the consensus primers GP5+/GP6+. The amplification of 20 µl PCR containing of 2xSYBR Green Supermix (Bio-Rad®), 50 pMol of each GP5+/GP6+ and 2 µl of DNA sample[12]. Amplifications were performed for 45 cycles using the following parameters; initial denaturation at 95 ºC 5 min, each cycle at 95 ºC for 10 s, 50 ºC for 15 s and 72 ºC for 15 s. The PCR product was verified by ethidium bromide staining after electrophoresis in 2% agarose gel on a Gel Doc XR system (Bio-Rad®).

Statistical analysis

Data was analyzed with the statistical software package SPSS (version 19). The chi-square test was used to analyze the correlation between anal cytology and HPV DNA testing. A value of $p<0.05$ was considered statistically significant.

Results

A total of 166 HIV-infected, there were 111 males and 55 females who were enrolled. The mean (SD) age at enrollment was 39.5±9.5 with a range of 17-67 years the most of them (46%) were older than 40 years in Table 1.

Among the 166 anal cytological specimens defined as normal was found in 118 (71.1%) and abnormal anal cytology was observed in 48 (28.9%). The most common of abnormal cytology was ASCUS 16.3%, followed by LSIL 11.4% and HSIL 1.2% (Figure 1).

The results of HPV testing for 166 samples were negative 103 (62.0%) and positive 63 (38.0%). The negative HPV DNA in normal, ASCUS, LSIL and HSIL were 68.6%, 59.2%, 31.6%, and 0%, respectively. The positive HPV DNA in normal, ASCUS, LSIL and HSIL were 31.4%, 40.8%, 68.4%, and 100%, respectively. The correlation of HPV DNA and anal cytology showed significant association with the presence of abnormal squamous cells intraepithelial lesions ($p<0.005$) in Table 2.

Table 1 General description of the patient study samples

Variables	Frequency (n= 166) (%)
Gender identity	
Male	111 (67)
Female	55 (33)
Age	
Mean ± SD (range)	39.5 ± 9.5 (17-67)
<20 years	1 (1)
20-29 years	22 (13)
30-39 years	67 (40)
≥ 40 years	76 (46)

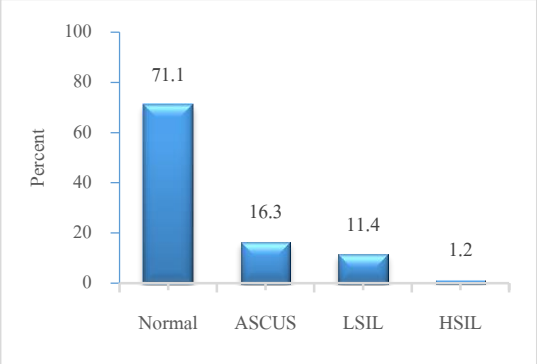

Figure 1 Distribution of anal cytological diagnosis

Table 2 Prevalence of HPV DNA in cytological diagnostic

Cytological diagnosis	All samples (n=166) no. (%)	HPV DNA status no. (%)	
		Positive	Negative
Normal	118 (71.1)	37 (31.4)	81 (68.6)
ASCUS	27 (16.3)	11 (40.8)	16 (59.2)
LSIL	19 (11.4)	13 (68.4)	6 (31.6)
HSIL	2 (1.2)	2 (100.0)	0 (0.0)

ASC-H and SCC not reported from this study

Discussion and Conclusions

HPV DNA normally found in anal intraepithelial lesion and anal squamous cell carcinoma and high incidence particularly among patient with HIV-infected (Assi et al., 2014). Several studies have reported the prevalence of anal HPV infection among HIV-infected individuals range from 16.0-85.0% (Gami et al., 2014; Gandra et al., 2015; Stier et al., 2015), while the previously reported range of abnormal anal cytology was 14.1-48.0% (Chaves et al., 2012; Darwich et al., 2013; Gandra et al., 2015). In anal sample collected with modified-LBC of our study demonstrated the prevalence of anal HPV infection and ASIL was 38.0% and 28.9%, respectively, which is within range. The prevalence of HPV infection and ASIL varies widely because of the study conducted in different regions, the studied population and the methodology using.

In our study, the cytological finding showed the most common anal abnormality was ASCUS (27 cases) which 16 of them were negative for HPV DNA. This may reflex the fact that the line of normal diagnosis was closely criteria to ASCUS that was consistent with benign reactive changes than malignancy (Abdul-Karim et al., 2015). The results of normal cytology were positive 37 cases of HPV DNA, because of high sensitivity of HPV DNA testing but the cellularity cannot yet change for correlated with intraepithelial lesion (Bean, Chhieng, 2010). The results of 19 LSIL cytology were negative 6 cases of HPV DNA, because the LSIL may be from other infection or causes. The results of 2 HSIL cytology were positive of HPV DNA both, which is persistent HPV infection. The HPV DNA testing is a high sensitivity but low specificity for screening cancer (Maia et al., 2013). Persistent HPV infection

observed frequently in immunocompromised patients that cannot analyzed by single testing (Tong et al., 2014).

In conclusion, modified-LBC (with 95% ethyl alcohol solution as the fixative) can be used for anal cancer screening by cytology and HPV testing. Our result showed that the anal cancer screening by HPV DNA testing and anal cytology using sample collected by modified-LBC have similar result to other studies and are effective to be used for screening anal precancerous lesion.

Acknowledgements

We would like to thank the HPV & EBV and Carcinogenesis Research Group, Khon Kaen University for supported the grant to this study.

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