

## RESEARCH ARTICLE

# Prevalence of Abnormal Anal Cytology in HIV-Infected Women: a Hospital-Based Study

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### Abstract

**Background:** To study the prevalence of abnormal anal cytology by Papanicolaou (Pap) technique in HIV-infected women who attended a HIV clinic at Prapokklao Hospital, Chanthaburi, Thailand. **Materials and Methods:** HIV-infected women who attended a HIV clinic at Prapokklao Hospital from March 2013 to February 2014 were recruited for anal Pap smears. Participants who had abnormal results of equally or over “abnormal squamous/glandular cells of undetermined significance” (ASC-US) were classified as abnormal anal cytology. **Results:** A total of 590 anal Pap smears were performed at HIV clinic of Prapokklao Hospital during the study period. There were only 13 patients who had abnormal Pap tests, which were: 11 ASC-US and 2 HSIL (high grade squamous intraepithelial lesion). The prevalence of abnormal anal Pap smears in HIV-infected women who attended HIV clinic at Prapokklao Hospital was 2.2 percent. Percentage of high risk HPV in patients who had abnormal Pap test was 88.9 (8/9). **Conclusions:** The prevalence of abnormal anal Papanicolaou smears in HIV-infected women who attended the HIV clinic at Prapokklao hospital was quite low in comparison to the earlier literature.

**Keywords:** Anal cytology - HIV - HPV - Thailand - international comparison

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### Introduction

Anal cancer is a disease in which malignant cells are formed in the epithelial tissue of the anus. It is a rare disease that incidence continued to increase by 0.3% per year from 2004 through 2008 in women but much less common than the cancer of the colon or rectum (Siegel et al., 2012). Known risk factors of anal cancer included human papillomavirus (HPV) infection (Ghosh et al., 2012; Afshar et al., 2013), human immunodeficiency virus (HIV) infection with low cluster of differentiation 4 (CD4) count, anoreceptive intercourse and cigarette smoking (Baranoski et al., 2012; Zhang et al., 2012). The American Cancer Society reported estimated new anal cancer cases in United States for 2014 were about 7,210 new cases and about 950 deaths (American Cancer Society, 2014). There was no previous report of specific anal cancer incidence in Thailand. It is commonly included in colorectal carcinoma incidence. The incidence is higher among women than among men in the general population (Kojic et al., 2011).

Invasive anal cancer arises from squamous intraepithelial lesion (SIL). SIL is likely to represent the precursor to anal cancer. Anal SIL (ASIL) ranges from low to high grade. High grade squamous intraepithelial lesion

(HSIL) is likely to represent the true invasive cancer-precursor lesion in the anal (Kojic et al., 2011). Low grade squamous intraepithelial lesion (LSIL) is clinically important because researches found LSIL progression into detectable anal HSIL in a short duration (Gaisa et al., 2014). Atypical squamous cells of undetermined significance (ASC-US) also may be found on cytologic examination in the anus accompanied by biopsy-proven SIL (Gaisa et al., 2014). European's study (Schim van der Loeff et al., 2014) showed that 80 percent of anal cancer caused by HPV especially in HIV-positive women.

So far there is no routine screening program for ASIL. In Thailand where HIV positive population is 1.1 percent in 2012 according to the World Bank. ASILs screening program could help to prevent anal cancer in a high-risk population. The aim of this study was to determine the prevalence of HPV-related abnormalities of the anal cancer in HIV-positive women by the use of cytology to see if the lesion risk factor could be found.

### Materials and Methods

This research was a prospective descriptive analysis. Prapokklao Hospital Ethic committee approved the study.

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It aimed to study the prevalence of the abnormal anal cytology in HIV infected women who came for a routine visit at HIV clinic of Prapokkklao Hospital, Chanthaburi, Thailand, between March 2013 and February 2014. Subjects filled written consent forms. Each then was given a self-administered questionnaire. Those that could not read proficiently were then interviewed instead. Physician in charge filled the form for this group.

Inclusion criteria were confirmed HIV infected women who visited Prapokkklao Hospital HIV clinic during the study period. Exclusion criteria were if patients had active lower gastrointestinal bleeding, anal inflammation, acute anal fissure and those who declined to participate in the research.

Anal Pap smear was collected using a long side of modified Ayre’s spatula (Chaves et al., 2012). It was inserted 2 cm into the anal canal. The spatula was rotated 360 degree in a cone shaped area and was then removed. Sample was then smeared onto a glass slide, and immediately fixed in 95% ethyl alcohol for 15 mins. Air dried samples were then transported to the laboratory and reviewed by cytopathologists.

Anal cytological results were classified as unsatisfactory for evaluation, negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesion (LSIL) or high grade squamous intraepithelial lesion (HSIL). Criteria used to evaluate cervical cytology belonged to the 2001 Bethesda System Terminology (Solomon et al., 2004).

Women who had abnormal anal cytological results were called back for further HPV testing. HPV testing was done using commercial kit of Cervista human papillomavirus high risk (HPV HR) assays (Hologic, Inc, Marlborough, MA, USA). If HPV HR testing showed positive for high risk HPV then the second step of HPV testing by commercial kit of Cervista HPV 16/18 assays (Hologic, Inc, Marlborough, MA, USA) was then used. This HPV testing utilized polymerase chain reaction (PCR) techniques according to manufacturer’s guidelines. Then they were referred to colorectal surgeon for further investigation.

Statistical analysis was performed by using Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS, Chicago, IL, USA). Frequency, percentage and the average mean were represented for demographic characteristics. Chi-square test was used to compare categorical data. A p-value less than 0.05 were classified as statistical significant.

**Results**

*Demographic and characteristic data on selected group*

There were 590 HIV infected-women in the present study. Subjects were all walks in patients who had regular visit at HIV clinic. Their ages ranged from 19 to 70 years, with average age of 41±8.8 years. The largest and second largest groups were the 30-39 and 20-29 years old at 39.3 and 36.4 percent, respectively. Only 3.6 percent had undergraduate degrees while the rest were primary and secondary school graduates. Subjects were

mainly of Thai nationality. They were two Cambodian nationals in the study who spoke fluent Thai. Fifty two percent were hired labor to the local business, 19.6 percent in agriculture and proprietary trade. Very few percent were housewives and government workers at 3.2 and 2.4 percent, respectively. Only 0.7 percent of participants (4/590) reported anal sexual intercourse habit. Eight of them (1.4%) had homosexual behavior. Other demographic and characteristic data of these participants was presented in Table 1.

*Prevalence of abnormal anal Pap smears*

Five hundred and seventy seven subjects had normal cellular appearance ( Figure 1). ASC-US was found in 11 patients as seen in Figure 2. Two cases showed HSIL of anal cytology as presented in Figure 3.

The prevalence of abnormal anal Pap smears in this study was 2.2% (13/590) as seen in Table 1. Two hundred and six cases (34.9%) were classified as inadequacy of specimen for evaluation. A total of 371 (62.9%) cases were in category of NILM (negative test). Atypical smears in this study were 1.9% (11/590). Only two cases (0.3%) had HSIL. No LSIL was found in this study. All abnormal anal cytology cases were forwarded to colorectal surgeon for further investigation and follow up according to standard hospital guideline.

**Table 1. Demographic Characteristics and Prevalence of Abnormal anal Cytology in the Population at Prapokkklao Hospital, Chanthaburi, Thailand**

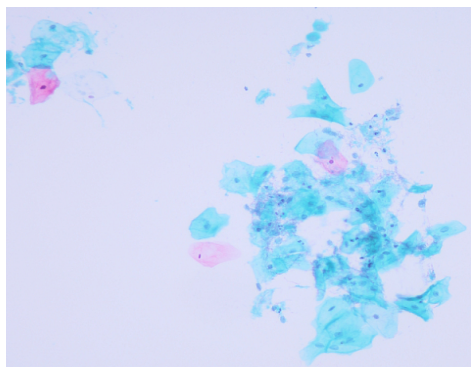
Characteristics	N=590 (%)	Anal cytology (%)		
		NILM	Atypical	HSIL
<b>Age (years)</b>				
<20	51 (8.6)	49 (8.5)	2 (18.2)	0
20-29	215 (36.4)	209 (36.2)	6 (54.5)	0
30-39	232 (39.3)	227 (39.3)	3 (27.3)	2(100)
40-49	92 (15.6)	92 (15.9)	0	0
<b>Occupation</b>				
Housewives	19 (3.2)	18 (3.1)	1 (9.1)	0
Agriculture/Trade	175 (19.6)	171 (29.6)	3 (27.3)	1 (50)
Government officer	14 (2.4)	14 (2.4)	0	0
Hired labors	307 (52)	300 (52)	6 (54.5)	1 (50)
High school/College student	3 (0.5)	3 (0.5)	0	0
Others	72 (12.2)	71 (12.3)	1 (9.1)	0
<b>Education</b>				
Primary school	557 (94.4)	544 (94.3)	11(100)	2(100)
Secondary school	12 (2.0)	12 (2.1)	0	0
At least undergraduate degree	21 (3.6)	21 (3.6)	0	0
<b>Marital status</b>				
Without partner	215 (36.4)	212 (36.7)	2 (18.2)	1 (50)
With partner	375 (63.6)	365 (63.3)	9 (81.8)	1 (50)
<b>Parity</b>				
Nulliparous	115 (19.5)	113 (19.6)	1 (9.1)	1 (50)
Multiparous	475 (80.5)	464 (80.4)	10 (90.9)	1 (50)
<b>Family Planning</b>				
No	164 (27.8)	161 (27.9)	3 (27.3)	0
TR	178 (30.2)	176 (30.5)	1 (9.1)	1 (50)
Condom	210 (35.6)	203 (35.2)	6 (54.5)	1 (50)
DMPA	25 (4.2)	25 (4.3)	0	0
OCP	13 (2.2)	12 (2.1)	1 (9.1)	0
<b>HAART</b>				
No	34 (5.8)	18 (3.2)	0	0
Yes	556 (94.2)	543 (96.8)	11(100)	2(100)

\*NILM: negative of intraepithelial lesion or malignancy; HSIL: high grade squamous intraepithelial lesion; TR: tubal resection, DMPA: depot medroxyprogesterone acetate; OCP: oral contraceptive pill, HAART: highly active antiretroviral therapy

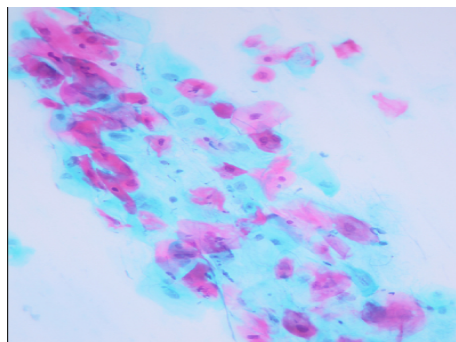
**Table 2. Prevalence Risk and 95% Confidence Interval (CI) of Abnormal Anal Cytology**

Characteristics	Anal cytology (%)		Prevalence Risk	95% CI	p value	
	NILM	Abnormal				
Age (years)	41+8.8	37+8.2		-1.079-8.606	0.12	
Duration of seropositive (years)	6+4.1	3+2.3		0.934-5.425	0.06	
Duration of HAART (years)	5+3.9	3+2.1		0.039-4.310	0.046	
Education	Primary school	544 (97.7)	13 (2.3)	0.266	0.088-0.802	0.12
	At least secondary school	33 (100)	0 (0)			
Marital status	Without partner	212 (98.6)	3 (1.4)	0.523	0.146-1.881	0.31
	With partner	365 (97.3)	10 (2.7)			
Anal intercourse	No	573 (97.8)	13 (2.2)			1
	Yes	4 (100)	0			
Smoking	No	570 (97.8)	13 (2.2)			1
	Yes	7 (100)	0			
HAART	No	18 (100)	0			1
	Yes	543 (97.7)	13 (2.3)			
CD4 (cells/cu.mm)	<200	62 (95.4)	3 (4.6)	2.349	0.664-8.316	0.17
	≥200	499 (98)	10 (2)			

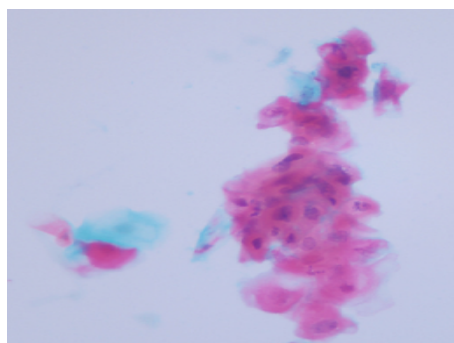
\*NILM: negative for intraepithelial lesion or malignancy; HAART: highly active antiretroviral therapy; CD4: cluster of differentiation 4



**Figure 1. Normal Anal Pap smear** (Papanicolaou Stain, Original Magnification 400x)



**Figure 2. Anal Pap Smear Showing Atypical Squamous Cell of Undetermined Significance** (Papanicolaou Stain, Original Magnification 400x)



**Figure 3. Anal Pap Smear of Anal High Grade Squamous Intraepithelial Lesion** (Papanicolaou Stain, Original Magnification 400x)

**Table 3. Anal Papanicolaou Smear Results in HIV-Infected Persons in Previous Literatures**

	Patarapadungkit	Dona	Chaves	Cheng	Greebon	Present study
Study year	2012	2012	2012	2014	2014	2014
Place	Thailand	Italy	Brazil	Taiwan	USA	Thailand
Cases (n)	177	346	184	230	470	590
Sex	MSM	MSM	Female	MSM	MSM	Female
Anal intercourse		66.1	62.5	58.7		0.7
Cytology	LBP*	LBP	CPP	LBP	LBP	CPP
Prevalence (%)	26	29.8	14.1	23	57.7	2.2
Inadequacy (%)	6.7		2.2			34.9
NILM (%)	66.7		83.7			62.9
ASC-US (%)	14.1	16.7	11.9	13.4	22.7	1.9
LSIL (%)	10.7	13.1	2.2	7	31.3	
HSIL (%)	1.2			2.6	3.7	0.3
Cancer (%)						

\*Modified by using 95% ethyl alcohol in place to PreservCyt solution (Cytoc, Marlborough, MA); HIV: human immunodeficiency virus, MSM: man who have sex with man; LBP: liquid-based Papanicolaou smear, CPP: conventional Papanicolaou smear; NILM: negative of intraepithelial lesion or malignancy; ASC-US: atypical squamous cell of undetermined significance; LSIL: low grade squamous intraepithelial lesion, HSIL: high grade squamous intraepithelial lesion

Comparison of demographic characteristics in women who had normal and abnormal anal cytology was presented in Table 2.

#### HPV DNA testing

All participants who had abnormal anal cytology result were called back for further investigation by HPV DNA testing. Only nine from thirteen cases underwent HPV DNA testing. Most of them (8/9) had positive results of high risk HPV testing that composed of HPV genotype: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. There were only one case (1/8) had positive HPV genotype 18.

#### Discussion

Currently, there was no screening program for anal cancer in Thailand. Anal cancer is a rare disease as a result there was no routine test. Anal region tissue structure is similar to that of cervicovaginal region. Pap smear is a worldwide screening tool for cervical precancerous and cancer condition. Colposcopic directed biopsy is the gold standard for diagnosis of women who had abnormal cervical cytology. HIV-infected women had high risk

of high risk HPV harbor. Pap smear is now the topic of interest for anal cancer screening due to similarity of cervical cancer.

Prevalence of abnormal anal cytology in presented study was 2.2 percent. This result was lower than those of previous literatures (Chaves et al., 2012; Dona et al., 2012; Patarapadungkit et al., 2012; Cheng et al., 2014; Greebon et al., 2014). Prevalence rate of abnormal anal cytology from various studies ranges from 23.0 to 57.7 percents (Dona et al., 2012; Patarapadungkit et al., 2012; Cheng et al., 2014; Greebon et al., 2014). These works were performed in HIV-infected men who had sex with men (MSM). Anal Pap smear in the work of Dona, Patarapadungkit, Cheng and Greebon were of liquid-based type. Liquid-based cytology was known to provide somewhat higher sensitivity than conventional Pap smear. The subjects in this study were of different cohort group. There were HIV-positive women while other studies paid attention to MSM. The anoreceptive intercourse in this study was 0.7 percent compared to 100 percent in MSM from other reports.

The work of Chaves et al., 2012 of Brazilian female showing the abnormal anal Pap smears prevalence rate of 14.1 percent. Anal cytology in Chaves' study was conventional Pap smear technique similar to the present study. Anal intercourse experience in Chaves' study was 62.5 percent (Chaves et al., 2012). Thai women had an embarrassment to anoreceptive intercourse conducts. Many subjects found that filling the questionnaire by themselves were overwhelming so they requested the physician to fill in the forms for them. We did not know for certain if the patients would tell the truth about their sexual act preference. It was easier to say that they were not involved in such a taboo subject than to have the fact recorded in writing with their names on it. These was a questionnaire filled in the hospital, the answer sheet would be kept with the patient record. This was not the anonymous questionnaire. The Thais were timid talking about sexual subject (Suwannarurk et al., 2009) for Thailand; an anonymous questionnaire system would give more details about sexual practice of subjects. However, under that condition one could not obtain any clinical data. The data comparison of this study and other literatures were summarized as shown in Table 3.

The duration of HIV seropositive in abnormal anal cytology and normal cytology were not different. This result was consistent with the Gingelmaier's work in year 2010 (Gingelmaier et al., 2010). Other demographic data such as education, marital status and sexual behavior as shown in Table 2 were not significantly different between normal and abnormal anal Pap smears.

High risk HPV in abnormal anal cytology in this study was 88.8 percent (8/9). This result was consistent with the work of Hoots in 2009 (Hoots et al., 2009). Hoots' work reported that the prevalence HPV type 16 and type 18 was 72 percent while the present study had only 1 case out of 8 or 12.5 percent. The limitations of this study were the tiny number of HPV testing and that the study time shortage may not provide the significant result. Larger sample size and additional of cervical cytology and long-term follow-up period were needed for future study.

In conclusion, the prevalence of abnormal anal Papanicolaou smears in HIV-infected women who attended HIV clinic at Prapokklao Hospital was quite low in comparison to other literature. High percentage of high risk HPV was found among the participants who had abnormal anal cytology. Anal Pap smears should be considered in the subjects who had positive high risk HPV.

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