RESEARCH ARTICLE

Self-Sampling Versus Physicians' Sampling for Cervical Cancer Screening - Agreement of Cytological Diagnoses

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Abstract

Background: A major problem with cervical cancer screening in countries which have no organized national screening program for cervical cancer is sub-optimal participation. Implementation of self-sampling method may increase the coverage. Objective: We determined the agreement of cytological diagnoses made on samples collected by women themselves (self-sampling) versus samples collected by physicians (Physician sampling). Materials and Methods: We invited women volunteers to undergo two procedures; cervical self-sampling using the Evalyn brush and physician sampling using a Cervex brush. The women were shown a video presentation on how to take their own cervical samples before the procedure. The samples taken by physicians were taken as per routine testing (Gold Standard). All samples were subjected to Thin Prep monolayer smears. The diagnoses made were according to the Bethesda classification. The results from these two sampling methods were analysed and compared. Results: A total of 367 women were recruited into the study, ranging from 22 to 65 years age. There was a significant good agreement of the cytological diagnoses made on the samples from the two sampling methods with the Kappa value of 0.568 (p=0.040). Using the cytological smears taken by physicians as the gold standard, the sensitivity of self-sampling was 71.9% (95% CI:70.9-72.8), the specificity was 86.6% (95% CI:85.7-87.5), the positive predictive value was 74.2% (95% CI:73.3-75.1) and the negative predictive value was 85.1% (95% CI: 84.2-86.0). Self-sampling smears (22.9%) allowed detection of micro-organisms better than physicians samples (18.5%). Conclusions: This study shows that samples taken by women themselves (self-sampling) and physicians have good diagnostic agreement. Self-sampling could be the method of choice in countries in which the coverage of women attending clinics for screening for cervical cancer is poor.

Keywords: Cervical scrapings; Self-sampling; Physicians' sampling; Cervical Cancer Screening; Pap smear Cytology

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Introduction

Self-sampling (SS) is a method to collect cervical specimens using a specially designed device to collect cervical cells at area of squamo-columnar junction of the cervix by the users themselves. There are many SS devices which have been clinically approved such as swabs, cervical brushes, tampon, and lavages (Schmeink et al., 2011). The collected materials taken from the SS devices are submitted to laboratories and treated as per routine samples for cytopathology examination and for HPV detection (Pengsaa et al., 2003, Okayama et al., 2012). The quality of SS samples is highly satisfactory even in elderly women (Tamalet et al., 2013). The SS method increases participation of non-responders in screening programs (Piana et al., 2011, Sancho-Garnier et al., 2013). It has been shown to have similar specificity and sensitivity to physicians sampling (Eperon et al., 2013, Karwalajtys et al., 2006, Harper et al., 2002, Forney et al., 2010). In one study in Lao PDR the acceptability of self-sampling is 62% compared to physicians sampling 36% (Yoshida et al., 2013)

There are various self-sampling devices in the market (Othman and Mohamad Zaki, 2014). The brush self-sampler is a type of device that need women to insert the bristles material into the vagina and is turned around to collect cells. Cytobrush is the most well-known brush tool to collect self-sampling materials. There are a variety of brush types SS devices such as Evalyn brush, Viba brush, and Femipap (van Baars et al., 2012). The samples taken can be transported dry or in transport medium (van Baars et al., 2012). Brushes are flexible and easy to use, can be processed in the same way as physician-obtained samples and are suitable for sending by mail (Schmeink et al., 2011). Self-collection using brush have been shown to have a higher sensitivity for cervical intraepithelial

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neoplasia grade two or worse than using Dacron or cotton swabs (Belinson et al., 2003, Szarewski et al., 2007, Gok et al., 2012b), and when given to non-responder in cervical cancer screening programme, it is non-inferior to the samples taken in the usual method (Bosgraaf et al., 2014).

The use of SS may lead to higher acceptability to screening (Gok et al., 2012a, Wikstrom et al., 2011). SS is more attractive for the non-attendees in countries which have organized screening and from rural women in countries which have limited resources (Sancho-Garnier et al., 2013). It might be an alternative method for women who are reluctant to undergo pelvic examination due to "shyness' (Scarinci et al., 2013) or too busy looking after the family which is often the case amongst Asian women (Othman and Rebolj, 2009). In addition, it may reduce the cost on the 'patients' and on 'hospitals' as no visits to clinicians are needed (Darlin et al., 2013, Scarinci et al., 2013). It is of interest to note that SS is also acceptable to men in the studies using self-obtained rectal specimens (Dodge et al., 2012). With minimal education on how to take the samples, the women can produce SS samples just as good as physicians' samples (PS) (Karwalajtys et al., 2006). The sensitivity and predictive value of HPV detection in SS samples is as good as PS samples (Cerigo et al., 2012, da Silva Rocha et al., 2012). There are studies which show SS samples yields more HPV positive results compared to PS samples (Cerigo et al., 2012, Karwalajtys et al., 2006, Agorastos et al., 2005).

In standard cytological testing, physicians' samples (PS) mainly contain endocervical and ectocervical cells, whereas SS samples are assumed to generally contain mixture of vaginal and cervical cells (Schmeink et al., 2011). The diagnostic sensitivity and specificity on SS samples are presumed to be lower, probably due to the fact that self-obtained samples mostly contain vaginal cells and not cells at the transformation zone of the cervix. With these issues in mind we embarked on a study on comparability in cytological diagnoses made from samples obtained by women themselves [The test method] with the samples taken by physicians [the gold standard].

Materials and Methods

We invited women volunteers to undergo 2 procedures; cervical self-sampling using the Evalyn brush (SS) and physicians sampling using cervix brush (PS). The step by step method is shown in Figure 1. The women were taught on how to do the SS method using video demonstration provided by the supplier. In addition, oral demonstration by using the SS device was also given by the doctor or the researchers. The SS samples were stored at room temperature and sent to pathology laboratory within 3 hours. Upon receiving in the laboratory, the brush material was separated from the casing and transferred into the methanol buffer solution as per the manufacturer's instructions (PreservCyt Solution, Cytyc, Boxborough MA). The samples taken by physicians were done in the usual conventional manner and treated as per routine testing. The smears from both methods were processed into Thin Prep monolayer smears and stained with Papanicolou stain. The smears were examined under light microscopy by pathologist [NHO] who was blinded of the method used. Statistical analyses were conducted using IBM SPSS Statistics 20. The overall agreement of SS smears and PS smears were tested using percentage (%) agreements and Cohen's Kappa statistics. Values of Kappa were categorized based on the agreement as follows; 0.0-0.2:poor, 0.21-0.40: slight, 0.41-0.60 moderate, 0.61-0.80 substantial, 0.81-0.90 almost excellent and 1.0 indicate excellent agreement. The level of significance was set at $p<0.05$.

The study protocol was approved by the University research Ethics Committee [ref no USMKK/PPP/JEPeM [242.3.(7)]

Results

A total of 367 women were included in the study, age range from 22 to 65 years age, with mean age of 40.7 years. The majority, 130 (35.4%) were 30-39 years of age. The characteristics of these women are shown in Table 1. In terms of adequacy of cervical sample material obtained by the two methods, there is significantly good agreement [Table 2]. On cytological examination (based on the Bethesda system classification), the comparability of the diagnoses is shown in Table 3. The percentage agreement

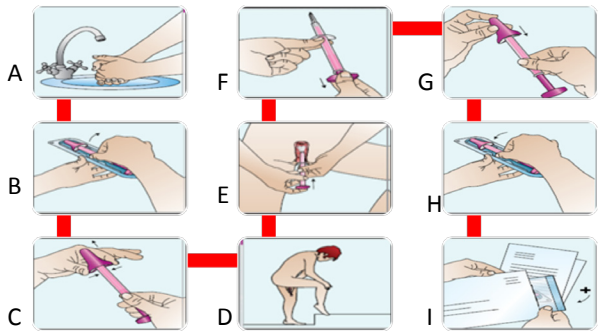


Figure 1. Procedures for Self-Sampling Method using the Evalyn brush [as provided by the manufacturer www.roversmedicaldevices.com]. Legends: A) Wash hands before usage, B) B. Remove the Evalyn brush from the packaging. Do not throw the packaging away, as it is necessary for sending the Evalyn brush to the laboratory after usage. C) Press the sides of the pink cap with your thumb and index finger to remove the pink cap from the Evalyn brush Ensure that you do not touch the white fibres of the Evalyn brush with your hands! D) Obtain the sample whilst in a standing position. Assume a comfortable stance (e.g. as if you were about to insert a tampon). E) Hold the transparent casing with one hand, and with your other hand, push the pink plunger in the direction of the transparent casing. You will hear and feel a click when the brush is in the right position with the pink plunger directly against the casing. F) Hold the transparent casing with one hand, and with your other hand, pull on the pink plunger until the white brush disappears into the casing. When doing so, do not touch the top part of the Evalyn brush above the wings. G) Hold the transparent end to ensure the white brush does not extend again. Place the pink cap back on the Evalyn brush using your thumb and index finger. You will hear a click when it is properly in place. H) Put the Evalyn brush back inside the packaging. I) Use the return envelope to send the plastic bag containing the Evalyn brush together with the signed declaration of consent

Table 1. Characteristics of Women Participating in the Self-Sampling Study (n=367)

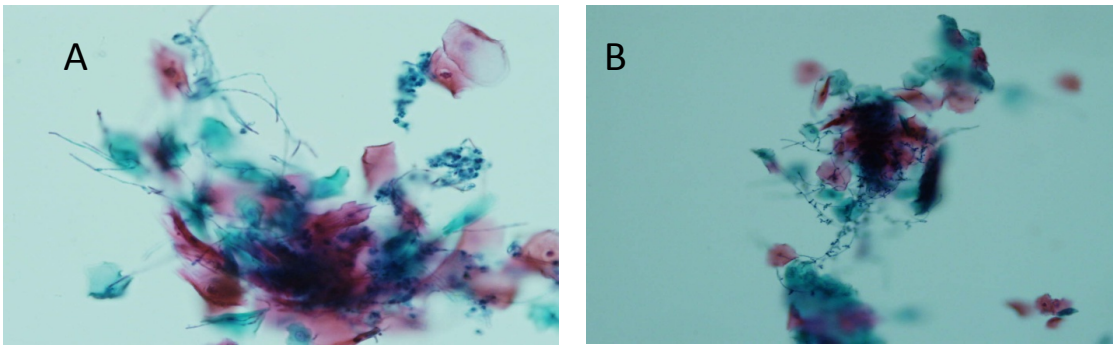
Characteristics	Number	%	Characteristics	Number	%
Age (years)			Marital status		
Mean	40.65		Single	1	0.3
Standard Deviation (SD)	9.251		Married	336	91.6
Median	40		Divorced	12	3.3
Range	22-65		Widowed	16	4.4
Missing data	3	0.8	No data	2	0.5
Age group (years)			Number of marriages		
20-29	44	12	0	1	0.3
30-39	130	35.4	1	330	89.9
40-49	120	32.7	2	28	7.6
50-59	64	17.4	4 and more	6	1.6
60 and above	6	1.6	(Missing)	2	0.5
Missing data	3	0.8			
Level of education			Age at the first marriage (years)		
No schooling	11	3	Mean	23.4	
Primary school	18	4.9	SD	3.996	
Secondary school	182	49.6	Median	23	
Certificate	34	9.3	Missing data	3	0.8
Diploma	78	21.3			
Degree	42	11.4			
Missing data	2	0.5			
Monthly income	99	27	Number of children		
RM0-999	69	18.8	0	19	5.2
RM1000-1999	62	16.9	1-2	93	25.3
RM2000-2999	60	16.3	3-4	150	40.9
RM3000-3999	28	7.6	5 and more	100	27.2
RM4000-4999	47	12.8	Missing data	5	1.36
RM5000 and above	2	0.5			
Missing data					
Menopausal status					
Yes	55	15			
No	310	84.5			
Missing data	2	0.5			

Table 3. Summary of Percentage Agreement of Cytological Diagnoses Made on Thin Prep smears by Physicians (PS) versus Thin Prep Smears by Self-Sampling (SS) Method (n=367)

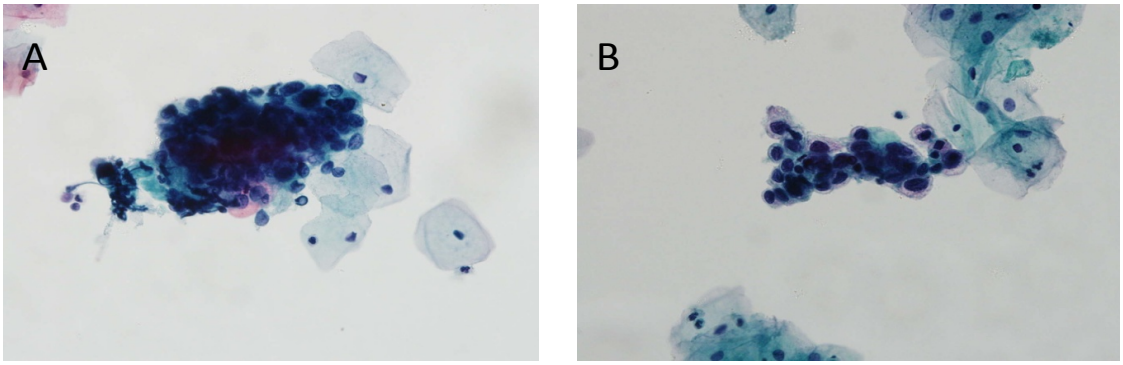
Category	Interpretation	PS (%)	SS (%)	Agreement (%)	Kappa Score	P-value
US	Unsatisfactory	12 (3.3)	22 (6.0)	6 (1.6)		
NILM	Negative	245 (66.8)	237 (64.6)	207 (56.4)		
Benign Cellular Changes	Candida spp	27	35	20		0.04
	Bacterial vaginosis	16	15	15		
	Actimyces spp	24	30	18	0.568	
	Trichomonas vaginalis	1	1	1		
Total infections		68 (18.5)	84 (22.9)	54 (14.7)		
Epithelial Cell	AS-CUS	5	4	2		
Abnormalities	ASC-H	0	0	0		
	LSIL	24	14	10		
	HSIL	4	1	1		
	AGC- (NOS) Endocervical cells	1	2	0		
	AGC- (NOS) Endometrial cells	4	2	2		
	AGC- (NOS) Glandular cells	2	0	0		
	AGC- (FN) Endocervical cells	2	1	0		
		0	0	0		
		0	0	0		
	Total abnormalities	42 (11.4)	24 (6.5)	15 (4.0)		
Total smears with similar results				282/367 (76.8)		

Legends: US, Unsatisfactory; NILM, Negative for intraepithelial or malignancy; AS-CUS, Atypical Squamous Cell of Undetermined Significance; ASC-H, Atypical Squamous Cell cannot exclude High Grade; LSIL, Low Grade Squamous Intraepithelial Lesion; HSIL, High Grade Squamous Intraepithelial Lesion; AGC, Atypical glandular cell; NOS, Non-otherwise specified; FN, Favor Neoplastic

was 76.8% with the Kappa score of 0.568. This suggests the level of diagnosis agreement between the smears taken by physicians (PS) and the smears taken by self-sampling (SS) was moderate and statistically significant, p=0.040 (Table 3). When the PS smears versus the SS smears were clustered together as "Normal" (all cases



Cytological Diagnosis of *Candida* Species



Cytological Diagnosis of High Grade Squamous Intra-Epithelial Lesion

Figure 2. Examples of Cytology Smears Taken by Physicians [A] and by Self-Sampling [B]

Table 2. Agreement for Satisfactory Sampling in Thin Prep Smears Taken by Physician (PS) versus Thin Prep Smears Taken by Self-Sampling (SS) Method

PS	SS		Total	Kappa	%
	US	SA	smears	score	Agreement
US	7	5	12		
	-1.90%	-1.40%	-3.30%	0.386,	94.6%,
SA	15	340	355	p-value	
	-4.10%	-92.60%	-96.70%	<0.001	
Total	22	345	367		
	-6.00%	-94.00%	-100%		

Legends: US, Unsatisfactory; SA, satisfactory

diagnosed as negative for epithelial cell or abnormalities) and "Abnormal" (all cases diagnosed as Benign Cellular Changes or higher) the percentage agreement improved to 81.5% and Kappa score of 0.589 (p=0.045). Using the cytological smears taken by physicians as the gold standard, the sensitivity of self-sampling is 71.9% (95% CI:70.9-72.8), specificity is 86.6% (95% CI:85.7-87.5) and the positive predictive value is (PPV) 74.2% (95% CI:73.3-75.1) and the negative predictive value (NPV) of 85.1% (95% CI: 84.2-86.0).

Examples of cytology diagnoses of these two methods are shown in Figure 2. Self-sampling was better at picking up micro-organisms compared to physicians sampling

Discussion

Pap smear cytology remains the gold standard for cervical cancer screening. For the samples to be worthy of screening, they must contain cells of the transformation zone; i.e. endocervical and/or metaplastic cells. This is the anatomical area where the glandular epithelium meets the squamous epithelium. The endocervical region is more difficult to reach on sampling especially in older women as the physiological transformation zones ascends slightly with age due to squamous metaplasia. When either of these cells is present the samples are deemed satisfactory or adequate. In our study, we observed 94.6% agreement in terms of adequacy of samples taken by physicians (PS) and by the women themselves (SS) (Table 2). This findings is fairly similar with results of studies using other self-sampling devices such as Kato device (Latiff et al., 2015a), swabs and tampons (Harper et al., 2002) and Fournier (da Silva Rocha et al., 2015). The kappa for agreement is significantly higher for younger than older women (Karwalajtys et al., 2006).

In terms of diagnostic comparability between smears taken by the two methods, the kappa agreement for our study is 0.568 (p=0.04) indicating that the level of agreement between smears taken by physicians (PS) and by self-sampling (SS) to be moderate and is statistically significant. Using cytological smears taken by physicians as the gold standard, the sensitivity of self-sampling in our study is 71.9%, specificity is 86.6% and the positive predictive value is (PPV) 74.2% and the negative predictive value (NPV) of 85.1%. Table 4 shows the comparability of abnormal cytology detection using different sample devices for the SS method and the PS method from several studies including ours. From this table, the sensitivity of the SS method in our study is better compared to four other studies but worse than another study done in Malaysia (Latiff et al., 2015a). However that study only had one positive and zero negative case. In general SS is acceptable, although perceived as inferior compared to PS method.

We observed that SS smears (22.9%) could detect micro-organisms better than physicians samples (18.5%) in our study. These findings were quite surprising to us. There are not many studies on the capability of SS method in detecting microorganisms (Nabandith et al., 2012, Pengsaa et al., 2003).

Self-sampling for cervical cancer screening may be the answer to increase participation of hard-to-reach women (Verdoodt et al., 2015). In Kelantan (a state in the north-eastern part of Malaysia) where this study takes place, is a relatively 'rural' place compared to other states in Malaysia. The population is relatively homogenous in terms of ethnicity as ethnic Malay forms 95% of the populations. Out of the 367 women we recruited into our study 88.8% of them were of ethnic Malay. The mean age was 40.7 which correspond to the age range of women who would have come forward to take pap smear screening. Many of these women had not done so and the subjects were keen on SS method. Most of the SS studies done previously were conducted in societies where the population is heterogeneous (Szarewski et al., 2007, Karwalajtys et al., 2006).

Malaysia is a developing country. There is no organised cervical cancer screening program available. Pap smear screening infrastructure still requires some improvement. The SS method is a new way for getting Pap smear which is new to Malaysian women. For accurate cervical cancer screening, regardless of the screening method, the following three points must be followed: (1) an appropriate sample must be collected; (2) the specimens must be prepared correctly; and (3) a cytotechnologist should be able to observe the specimens (Okayama et al., 2012). SS samples satisfy these requirements. Offering SS to non-attendees opportunistically in primary care is feasible (Lim et al., 2016).

Self-Sampling is not without limitations. Using this method alone in screening for cervical cancer may deprive women of pelvic examinations which are usually done by physicians before the procedure. Lack of confidence in SS results is the most common reason why women prefer PS method (Guan et al., 2012). The SS devices are not customized to slight anatomical variation of female genital tracts. The transformation zone area in elderly women is higher than younger women thus may be difficult to reach giving rise to unsatisfactory or inadequate samples. There is also a potential risk of traumatizing and perforating the mucosa of the vagina and cervix in the process of getting the samples in women who do not follow proper instructions. In order for SS to be successful, the women must have a minimal level of education in order to read and understand the manual (Forrest et al., 2004). They need to clearly follow the instruction in order to get satisfactory samples. Some women complained that they have difficulty to understand the instruction because of the medical terminology used in the pamphlet (Howard et al., 2009). Some women especially those who have never used tampon may have anxiety to insert the device.

In conclusion, This study shows that cervical samples taken by women themselves (self-sampling) and by physicians have good diagnostic agreement. Self-sampling could be the method of choice for women who are not coming forward for pap smear screening for whatever reasons. It could also be used to increase coverage of women being screened against cervical cancer in those countries which have organized national programs.

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