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CERVICAL CANCER SCREENING OVERVIEW USING LIQUID-BASED CYTOLOGY (LBC) AND HUMAN PAPILLOMAVIRUS (HPV) DETECTION IN YOGYAKARTA

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ABSTRACT

Background: Screening of cervical cancer persists as a critical approach to persevere as a consequence of the gradually increasing cases and causing high mortality in women, including in Indonesia. The liquid-based cytology (LBC) and human papilloma virus (HPV) is a combination technique which promising and prosperous in detecting cervical cancer. **Objective:** The study aims to overview the cervical cancer screening results clinically using LBC and HPV testing in Yogyakarta. **Methods:** secondary data obtained from the medical records of 100 participants who participated in cervical cancer screening. The data collected included sociodemographic, clinical condition overview of the cervix, and results of LBC and HPV tests. **Results:** The sociodemographic data showed the housewives being a higher occupation percentage (59.8%), and the intrauterine devices (IUD) were the widely used types of contraception (42.3%). The screening was dominantly attended by young age (85.6%). The LBC result showed all of the participants had NILM types (100%), while the HPV results obtained five participants (5.2%) with positive results. In those positive results also discovered the bacterial vaginosis and reactive cellular changes associated with inflammation ($p \leq 0.05$). **Conclusion:** This study concluded that the result of screening cervical cancer in Yogyakarta revealed normal cases in the NILM condition based on the LBC test result. However, the HPV test indicates sensitivity properties from the capability to detect HPV in asymptomatic participants. Thus, the LBC and HPV testing are very suitable and effective to use as a combination for cervical cancer screening strategy.

INTRODUCTION

Cervical cancer occupied fourth place as a common type of cancer in women globally after breast cancer, lung cancer, and colorectal cancer, mainly caused by human papillomavirus (HPV). Some risk factors also contribute, including infection by a virus (ex, human deficiency virus (HIV)) or bacteria (*Chlamydia trachomatis*) due to unsafe sex, long-term usage of oral contraceptives, a greater number of labour deliveries, marital status, and smoking[1,2]. The incidence of new cases in 2022 is about 661,021 and 348,189, causing mortalities worldwide[3]. The progressivity risk of cervical cancer remains high in countries with low Human Development Index (HDI) levels, which are about three times higher, together with the mortality rates six times higher, than in high HDI level countries[4].

The National Statistic Agency (BPS)[5] stated that Indonesia's HDI achieved a score of 75.02. Based on the Human Development Report (UNDP) classification, the score is considered a high HDI level. However, cervical cancer in Indonesia ranked second among cancers occurring in women, with the number of cases of 36,964 (16.8%) and causing death by 20,708 (8.5%) in 2022[6]. Some factors contributing to the development of cervical cancer are smoking (2.6%), high fertility rate (2.3 live births per woman), hormonal contraception usage (12.3%), and HIV

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(0.3%)[7]. In Yogyakarta, the latest data obtained the incidence and prevalence of cervical cancer were more than 20 per 100,000, and the mortality rate was approximately 12 per 100,000 in 2017[8].

The World Health Organization has established elimination targets of cervical cancer for 2030, including 90% of girls aged 15 years old receiving full vaccination of HPV, 70% of women screened twice by age of 35 years and again by age of 45, and 90% of women suffering from cervical cancer disease receiving treatment[9]. Screening for cervical cancer in Indonesia has been introduced since 2008, and until 2019, resulted in only 12% of women aged 30-49 years having been screened[7-9]. That percentage is still far from the WHO recommendation. Besides, Indonesia still uses Visual Inspection with Acetic Acid (VIA) as the primary cervical cancer screening test[9]. The modality of VIA for cervical cancer screening has an advantage in high sensitivity and might reduce mortality, but the shortcomings such that unaffected by the incidence of invasive cervical cancer, and the effectiveness is unconvincing and has less specificity[10,11].

Human Papillomavirus (HPV) test is a promising modality for cervical cancer testing because of higher sensitivity and specificity than conventional cytology and Pap smear[12-14]. However, the HPV test cannot stand alone because it requires women to undergo colposcopy in large quantities, leading to an overload of colposcopy services. Therefore, the HPV test needs another referral triage method, such as liquid-based cytology (LBC)[13]. LBC would be combined with the HPV test because of the suitability of cellular residue[15]. The study aimed to obtain an overview of cervical cancer screening results clinically using LBC and HPV testing in Yogyakarta, as further used as a preliminary screening.

METHODS

Design Study

The study was a cross-sectional study using secondary data from the medical records of participants who had been followed up for cervical cancer screening at Tegalrejo health center, Yogyakarta, in July 2023. This was selected as a research location because the health services covered a wide range of regions, including four urban villages and 188 neighbourhoods. In those periods, 100 samples were targeted to participate in the program.

Procedure

Data obtained from medical records included sociodemographic (age, occupation, and contraception usage), clinical condition overview of the cervix, and results of LBC and HPV tests. Participants' criteria were included in this study, as shown in Figure 1. The inadequate data or samples were excluded from the study. The sampling process was conducted by local health workers and midwives. Sample retrieved from the ectocervix or endocervix using Cytobrush. The Cytobrush was next divided into two parts and placed in liquid preservative (ThinPrep, PreservCyt Solution(R)-Hologic, Inc., Marlborough, MA, USA)

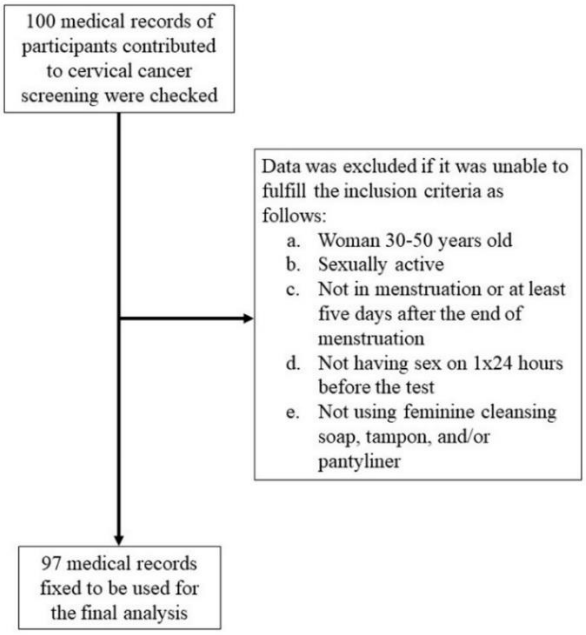

Figure 1. Cervical cancer screening flowchart for LBC and HPV diagnosis

HPV and LBC Laboratory Detection Methods

The HPV test was carried out using the HPV-DNA kit according to Andrijono et al.[16]. Samples were homogenized by centrifugation at 8000 rcf 10 min. Pellet was taken and performed resuspension. Next, DNA samples were extracted using a spin column. Eluent DNA 5µL mixed with 15 µL CerviScanReadyMix diagnostic kit (CerviScan, Bio Farma). Later, amplification was conducted using a BioRad CFX-96 thermocycler with 45 cycles. The amplification signal was detected when the Ct value was ≤ 40. The Ct value will be shown for HPV types, such as HPV 16 (HEX), HPV 18 (Texas Red), HPV

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52(Cy5), dan HPV without a specific type (FAM). Undetected internal control indicated an invalid result.

The LBC test was performed according to the method previously described by Makde and Sathwe[17]. A filtered cylinder was placed into a vial ThinPrep contained sample, and later inserted into the ThinPrep Processor. Then, the sample suspension was aspirated using a vacuum. The cell debris would be passed through the filter, whereas the target sample would be attached to the external surface of the filter. The filtered cylinder was released from the vial, and the filter part with the samples was transferred into a glass slide. The sample was stained using Papanicolaou staining. The LBC results were interpreted based on the Bethesda System (TBS) 2014[18]. This provides three general categories for diagnosing cervical cancer, as follows: Negative for Intraepithelial lesion or malignancy (NILM), Epithelial cell abnormalities: specify squamous or glandular, and others[18].

Statistical Analysis

Data analyzed as a descriptive statistical test using SPSS 20.0. Continuous variables were expressed as means and standard deviation (SD), while categorical variables were expressed as frequency and proportion[19]. Significant differences were statistically considered with a p-value ≤ 0.05.

RESULTS

A total of 97 participants were included in this study from the initial target of 100. Three samples were excluded because of incomplete acquired data (one sample did not have adequate samples to analyze in LBC and HPV, and cervical condition data of two samples were empty). The sociodemographic, LBC, and HPV prevalence of 97 participants enrolled in this study are shown in Table 1.

Table 1. Sociodemographic, LBC, and HPV characteristics of cervical cancer participants in Yogyakarta.

No.	Variables	Sub-variables	Results
1.	Occupations	Housewives	58 (59.8%)
		Health workers	3 (3.1%)
		Educational staffs	2 (2.1%)
		Employees	20 (20.6%)
		Freelancers	10 (10.3%)
		University students	1 (1%)
		Unemployed	3 (3.1%)
2.	Contraception usage	Intrauterine device (IUD)	41 (42.3%)
		None	38 (39.2%)
		Tubectomy (MOW)	8 (8.2%)
		Implant	3 (3.1%)
		Oral pills	1 (1%)
		Hysterectomy	1 (1%)
		Condom	2 (2.1%)
		Sterile	1 (1%)
		Injection	2 (2.1%)
3.	Age	25-50	83 (85.6%)
		51-65	14 (14.4%)
		Total	**97 (100%)**
		Age minimum	27
		Age maximum	62
		Mean ± SD	41.38 ± 8.43
4.	LBC test results	NILM	97 (100%)
5.	HPV test results	Positive	5 (5.2%)
		Negative	92 (94.8%)

More than half of the occupations were predominantly housewives (59.8%). Most of the contraception types used were intrauterine devices (IUD) (42.3%). Those percentages were not quite different from participants that was not using any contraceptive devices (39.2%). From an age perspective, participants at young were more common than older, which the youngest being 27 years old, while the oldest was 62 years old.

All participants had NILM types in LBC test results (100%) (Table 1), with sub-categorised results can be seen in Figure 2.

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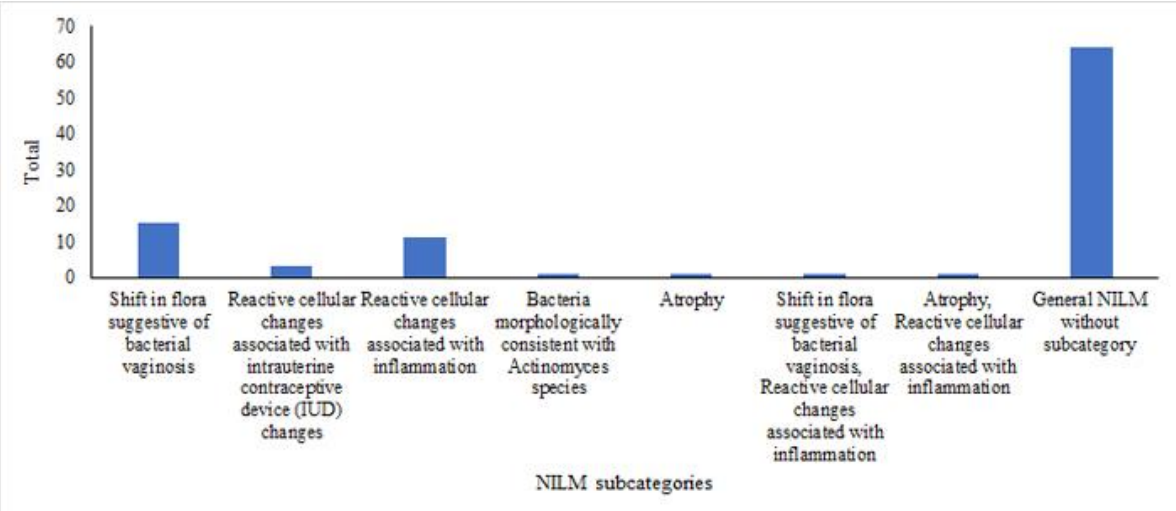

Figure 2. NILM subcategories the results of participants screened for cervical cancer

A total of 64 samples (66%) were considered as general NILM without any specific subcategories. Subcategory of shift in flora suggestive of bacterial vaginosis has a relatively high number, as well as 15 samples (15.5%). Next, subcategories sequentially towards a small samples size were reactive cellular changes associated with inflammation (11 samples or 11.3%), reactive cellular changes associated with intrauterine contraceptive device (IUD) changes (3 samples or 3.1%), bacteria morphologically consistent with actinomyces species, shift in flora suggestive of bacterial vaginosis, reactive cellular changes associated with inflammation, atrophy, and atrophy with reactive cellular changes associated with inflammation. The last five subcategories shared the same value (1 sample or 1%) each.

The HPV test detected 5 samples (5.2%) with positive results (Table 1), with the types of HPV presented in Figure 3. Three samples (3.1%) were found to have HPV type 52, and two (2.1%) had unknown specific types.

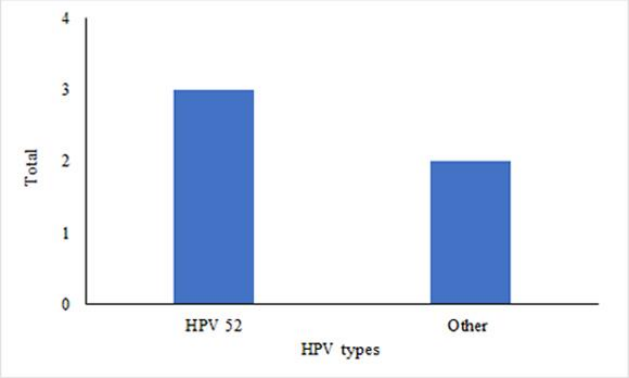

Figure 3. HPV types detected in participants' samples screened for cervical cancer

The prevalence of cervical conditions was 71 participants in normal condition (73.2%), whereas 23 participants were diagnosed with cervical erosion (23.7%), two participants had polyps (2.1%), and one participant (1%) had a mass located in the cervical os (Figure 4).

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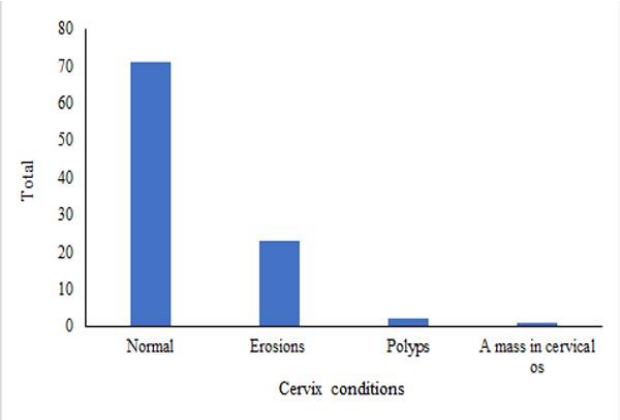

Figure 4. Cervical alteration participants screened for cervical cancer

One participant reported a condition of HPV positive with bacterial vaginosis (20%). Another participant with HPV positive has multiple cervical conditions, which, besides bacterial vaginosis, also entail reactive cellular changes associated with inflammation (20%). The remaining participants with HPV positive did not indicate any specific cervical alteration (3 samples or 60%). However, overall prevalence between NILM subcategories with HPV results demonstrated a significant value ($p \leq 0.05$) (Table 2).

Table 2. Interconnections between NILM subcategories and HPV test results of participants screened for cervical cancer

No.	NILM subcategories	HPV test result		p-value
		Positive	Negative	
1.	Shift in flora suggestive of bacterial vaginosis	1 (20%)	14 (15.2%)	0.007
2.	Reactive cellular changes associated with intrauterine contraceptive device (IUD) changes	0 (0%)	3 (3.3%)	
3.	Reactive cellular changes associated with inflammation	0 (0%)	11 (12%)	
4.	Bacteria morphologically consistent with Actinomyces species	0 (0%)	1 (1.1%)	
5.	Atrophy	0 (0%)	1 (1%)	
6.	Shift in flora suggestive of bacterial vaginosis, Reactive cellular changes associated with inflammation	1 (20%)	0 (0%)	
7.	Atrophy, Reactive cellular changes associated with inflammation	0 (0%)	1 (1%)	
8.	General NILM without subcategory	3 (60%)	61 (66.3%)	
Total		**92 (100%)**	**5 (100%)**	

Through the acquisition of 97 participants, both on general NILM without any subcategories or negative HPV test results, predominantly connected with normal cervical conditions, which are 50 samples (70.4%), and 68 (95.8%), respectively (Table 3). A leftover was undergoing an abnormal cervical condition, such as ectropion or erosion, polyp, or a mass in the cervical os. NILM subcategories of shift in flora suggestive of bacterial vaginosis and reactive cellular changes associated with inflammation were found in cervical erosion, with the same number (4 or 17.4%). A small quantity of NILM subcategories (1 sample each or 50%) presented polyps or a mass in the cervical os. For the HPV tests, five participants with positive results showed three samples (4.2%) with a normal cervix, and the rest had cervical erosion. (Table 3). However, the connection between NILM subcategories and cervical conditions, along with HPV test results and cervical conditions, did not yield significant results ($p = 0.928$ and $p = 0.831$, respectively).

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Table 3. Interconnections between NILM subcategories, HPV test results, and cervical alteration of participants screened for cervical cancer

Variables	Cervical condition				p-value
	Normal	Erosion	Polyp	A Mass in the cervix os	
A. NILM subcategories					
1. Shift in flora suggestive of bacterial vaginosis	10 (14.1%)	4 (17.4%)	1 (50%)	0 (0%)	0.928
2. Reactive cellular changes associated with intrauterine contraceptive device (IUD) changes	2 (2.8%)	1 (4.3%)	0 (0%)	0 (0%)	
3. Reactive cellular changes associated with inflammation	6 (8.5%)	4 (17.4%)	1 (50%)	0 (0%)	
4. Bacteria morphologically consistent with Actinomyces species	0 (0%)	1 (4.3%)	0 (0%)	0 (0%)	
5. Atrophy	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)	
6. Shift in flora suggestive of bacterial vaginosis, Reactive cellular changes associated with inflammation	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)	
7. Atrophy, Reactive cellular changes associated with inflammation	1 (1.4%)	0 (0%)	0 (0%)	0 (0%)	
8. General NILM without subcategory	50 (70.4%)	13 (56.5%)	0 (0%)	1 (100%)	
Total	**71 (100%)**	**23 (100%)**	**2 (100%)**	**1 (100%)**	
B. HPV test results					
1. Positive	3 (4.2%)	2 (8.7%)	0 (0%)	0 (0%)	0.831
2. Negative	68 (95.8%)	21 (91.3%)	2 (100%)	1 (100%)	
Total	**71 (100%)**	**23 (100%)**	**2 (100%)**	**1 (100%)**	

DISCUSSIONS

Our current study demonstrated that the LBC results of all participants have been categorized as NILM, indicating a normal cervical condition[20]. However, HPV testing generated a positive outcome in five participants (5.2%). Specific HPV results appeared as type 52, which is categorized as a high-risk HPV genotype. The HPV 52 types were found to be as highest genotype distributed in Southeast Asia, including Indonesia[21,22]. The difference in the result of the LBC and HPV test can be correlated with the sensitivity and specificity properties based on the previous studies[23-25]. The LBC usage for primary detection of cervical cancer combined with HPV triage or vice versa demonstrated balance in sensitivity and specificity when deployed for Atypical Squamous Cells of Undetermined Significance (ASC-US), continued with detecting CIN cervical intraepithelial neoplasia grade 2 and 3 (CIN2+, CIN3, and CIN3+) endpoints compared with LBC or HPV alone[23-25]. Besides, women only infected with HPV indirectly would develop cervical cancer. Largely, the HPV infection disappears within one or two years, except for some proportion that remains persistent and becomes a risk factor for resulting cervical cancer[26]. Those become another reason for the HPV test being insufficient to use alone, and as a factor of our HPV test detected a few results in participants. The stronger persistence tends to be detected in women with CIN2-3/HSIL+ than NILM, ASC-US, and Low Squamous Intraepithelial Lesion (LSIL), which often regress[27-29].

The acquisition of NILM has found multiple types of nonneoplastic cellular circumstances subgroups based on the Bethesda System[18], which appear to have high results comprised of linkage with organisms, leading shift in flora suggestive of bacterial vaginosis, followed by reactive cellular changes specific to inflammation. Bacterial vaginosis and inflammation were recognized as numerous conditions found in

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cervical cancer screening according to the previous study[30-32]. Contrary to our research, those studies showed inflammation had a more significant result than bacterial vaginosis (Table 4). The differences are affected by multiple factors, such as demographics and etiological conditions[33].

Table 4. Comparison with previous studies of bacterial vaginosis and inflammation resulted from the LBC test result

No.	NILM sub-category	Recent study (100 samples)	Meghana et al. [30] (1500 samples)	Jain et al. [31] (760 samples)	Afzal et al. [32] (230 samples)
1.	Shift in flora suggestive of bacterial vaginosis	15 (15.5%)	47 (3.13%)	190 (25%)	16 (6.9%)
2.	Reactive cellular changes associated with inflammation	11 (11.3%)	1134 (75.6%)	47 (6.18%)	121 (52.1%)

In contrast with our study, which did not obtain specific bacteria unless Actinomycetes, multiple types of bacterial species have been found, such as Trichomoniasis and Candidiasis[30-32]. However, our result showed that a bacterial vaginosis condition has HPV HPV-positive test result, which one combined with an inflammatory circumstance. According to previous studies mentioned, bacterial vaginosis was related to the risk of HPV infection and provides a potential in developing cervical cancer[34-36]. A study in China by Li et al.[34] found bacterial vaginosis and HPV infection high risk significantly increasing atypical squamous cell of undetermined significance (ASC-US), which be able to be rising trend in squamous cervical cancer (SCC). Other explanations showed co-infection among some bacterial agents of bacterial vaginosis, such as *Gardnerella vaginalis, Megasphaera* type I, and Clostridia-like bacterial vaginosis-associated bacteria 1 with high-risk HPV affecting the development of low-grade (LSIL) and high-grade (HSIL) squamous intraepithelial lesions[37]. Those studies confirmed by Nayak et al.[35] in case of positive result of bacterial vaginosis outreach a high trend of cervical intraepithelial neoplasia (CIN) incidence. The CIN occurrence, mainly CIN-2 and CIN-3, also presented in women with reactive cellular changes (RCC) within 7 years[38].

Cervical erosion was discovered in the general NILM subcategory or normal condition, followed by bacterial vaginosis, RCC associated with inflammation and intrauterine contraceptive device (IUD) changes, and bacteria morphologically consistent with Actinomyces species. Several factors affected erosion in the cervix, such as hormonal fluctuation, bacterial pathogens, inflammation, and foreign bodies, such as contraceptive device usage[39,40]. In case of hormonal fluctuation, a study by Banouei and Tahamtan[41] found an increase in estrogen levels correlated with the presence of cervical erosion. That's possible, contributing to our research which cervical erosion was discovered through a normal lesion result. Cervical erosion is possible, which provides a risk in generating cervicitis, allows in presenting HPV infection, and supports to development of cervical cancer[42,43]. The HPV test result of our study was also correlated with cervical erosion. This result was supported by a previous study, which stated the incidence of HPV could be found in asymptomatic conditions, and cervical erosion apparently has a risk of emergence of HPV[44].

A small number of cervical conditions were found to have a polyp and a mass at the cervical os. Cervical polyp is recognized dominantly as asymptomatic, usually benign, and have a tendency more premalignant than malignant lesions [45,46]. This possibility was affected by hormonal or genetic alterations; while a rare case was caused by IUD contraception, causing inflammation or infection[45,47]. Those conditions, at worst, are continuing with bacterial vaginosis, aggravating the cervical inflammation, to over proliferation in the repairing process after inflammation, which gives the possibility to develop symptomatic and malignant conditions because of tumour growth risk[42,45]. It aligned with our data in Table 3, which cervical polyps were found in bacterial vaginosis and RCC associated with inflammation. Besides, one sample with a mass at the cervical os did not get clear data, resulting in being assumed as multiple diagnoses, such as the polyp itself, cervical stenosis, Nabothian cyst, leiomyoma, or endometriosis[48].

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The limitation of this study is that our smaller sample size becomes the main reason the LBC and HPV test results obtained not specific and heterogeneous categories, thus larger samples are required to enhance the reliability of the results. Besides, the absence of histopathological or colposcopy examination, as the LBC test results were normal, and the clinical presentation did not indicate malignancy. Therefore, we are also unable to determine whether patients with positive HPV findings in this study exhibited any epithelial changes or malignant transformation.

Further research suggested focusing on boarder demographic to obtain findings and improve screening programs. This technique could be considered as VIA method replacement for implementing effective screening programs and reducing the burden of cervical cancer. In addition, our results of the HPV test indicated that all participants were positive for 52 HPV types, supporting the previous study[22]. Therefore, we should be provided with vaccine options suitable for Indonesian women against cervical cancer, which is a non-covalent vaccine (Gardasil-9).

CONCLUSION

Our study concludes that the result of screening cervical cancer in Yogyakarta revealed normal cases with NILM condition based on the LBC test result. However, the HPV test indicates sensitivity properties from the capability to detect HPV in asymptomatic participants. The lack of the HPV test is low in specificity and tends to regress in cases of NILM, LSIL, and AS-CUS. Thus, the LBC test is useful to enhance the specificity and cervical screening results. Therefore, the LBC and HPV testing are very suitable and effective to use as a combination for cervical cancer screening strategy.

ETHICAL APPROVAL

This study has been approved by the Medical and Health Research Ethics (MHREC) Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada-Dr. Sarjito General Hospital no. KE/FK/0837/EC/202.

CONFLICTS OF INTEREST

All authors declare no conflict of interest for this study.

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AUTHOR CONTRIBUTIONS

Conceptualization, ASBS; methodology, ASBS, FDT, EKD; validation: AZ, IW; formal analysis, FDT, AZ, EKD; investigation, FR; data curation, FR; writing—original draft preparation, ASBS, FR; writing—review and editing, FDT, AZ, EKD, IW; supervision, ASBS, IW; funding acquisition, ASBS.

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