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The Clinical Value of Screening for Cervical Cancer using the High Risk Human Papilloma Virus (HR-HPV) and Liquid Based Cytology (LBC) Co-Testing Approach Compared to Screening with Individual Tests in Harare, Zimbabwe

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Authors' contributions

This work was carried out in collaboration among all authors. Author RC wrote the protocol and wrote the first draft of the manuscript. Author TD managed the statistical analysis. Authors EM and LWM review of first draft of the manuscript. Author CN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Cervical cancer is the most common malignancy in Zimbabwe. This attributed to poorly executed Pap smear based screening programs. Pap smears have poor sensitivity and high specificity while HR-HPV DNA testing has high sensitivity and poor specificity. With reference to the ASCCP guidelines, does combining these two tests have any clinical value in terms of increasing

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screening intervals from 3 to 5 years, predicting possible future cervical lesion and resolving the dilemma associated with managing patients with borderline ASCUS LBC results.

Aim: To assess the clinical value of LBC and HR-HPV DNA co-testing in terms of increasing screening intervals (in the NILM/HPV- group), predicting possible future cervical lesions (in the NILM/HPV+ group) and the resolution of the dilemma associated with managing patients with borderline ASCUS LBC results.

Study design: Cross sectional descriptive.

Place and duration of study: Cimas Medical Laboratories. February 2020 to November 2020.

Methodology: A Thin Prep 2000 machine was used to process the LBC samples. The slides were then stained using the Papanicolaou stain. Two independent Cytologists interpreted the LBC smears according to the 2014 Bethesda System. Discrepant results were resolved by an independent Pathologist. All interpreters were blinded of the HR-HPV test result. HR-HPV DNA testing was done using the Cepheid Xpert HPV qualitative test.

Results: A total of 542 women of ages' ≥ 30 years were recruited into the study. The mean (SD) of the women was 39.4 (8.6) years, the median age was 37 years and the age range was 30-83 years. The mean ages' of the HR-HPV+ and HR-HPV- were comparable (40.1 years vs. 38.9 years, respectively; p=0.412). LBC abnormalities were significantly higher in women >37years (median age) than women ≤37 years (18.2% [4/22] vs. 81.2% [18/22], respectively; *p*=0.002).The LBC/HR-HPV test combination results were as follows: NILM/HPV- (n=418, 77.1%), NILM/HPV+ (n=102, 18.8%), ASCUS/HPV+ (n=9, 1.7%), ASCUS/HPV- (n=5, 0.9%) and >ASCUS/HPV+ (n=8, 1.5%).

Conclusions: The co-testing approach had the clinical value of increasing screening intervals from 3 to 5 years in 77.1%, predicting possible future cervical lesion in 18.8%, instant confirmation of a precancerous lesion from ASCUS lesions in 1.7% and the instant confirmation of a non-neoplastic lesion from ASCUS lesions in 0.9% of all co-tested samples.

Keywords: Human papillomavirus; liquid based cytology; cervical cancer; visual inspection with acetic acid; atypical squamous cells of undetermined significance.

ABBREVIATIONS

LBC : Liquid Based Cytology
HPV : Human Papillomavirus
ASCUS : Atypical Squamous Cells of Undetermined Significance
LSIL : Low Grade Squamous Intraepithelial Lesion
HSIL : High Grade Squamous Intraepithelial Lesion
DNA : Deoxyribonucleic Acid
ASCCP : American Society of Colposcopy and Cervical Pathology

1. INTRODUCTION

Cervical cancer is ranked as the 3rd most common malignancy in women and is the 4th leading cause of mortality in women worldwide [1]. However, in Zimbabwe, cervical cancer is the most common malignancy regardless of gender. Over 5000 new cancer cases are diagnosed annually and approximately 35% of these are cervical cancers [2]. In Zimbabwe, cervical cancer accounts for 13% of the 3500 cancer related deaths [2].

In developed countries, the incidence of cervical cancer plummeted over the past seven decades after the introduction of the Papanicolaou (Pap) smear in 1949 [3]. However, the incidence of cervical cancer is still very high in developing countries like Zimbabwe [3]. This is attributed to poorly executed population based cervical cancer screening programs. Despite having helped to reduce cervical cancer burden, Pap smears are not perfect as their sensitivity is affected by sampling and interpretation errors [3]. Liquid based cytology (LBC) is the newer cervical smear technique on the market that permits performance of HR-HPV DNA testing on the same sample, the immediate fixation of cells and the production of a cleaner preparation which increases screening efficiency.

The establishment of the cause – effect between HR-HPV infection and cervical cancer prompted the introduction of HR-HPV DNA testing as a primary screening test for cervical cancer [4].The advantages for HR-HPV DNA testing are high reproducibility, high sensitivity and high negative predictive value (NPV) [5]. However, the major limitations for HPV DNA testing are low positive predictive value (PPV) and poor correlation with clinical disease [5]. A positive HPV result does not always signify the presence of a treatable cervical lesion [5]. Thus, HPV results should be interpreted with caution.

HPV has higher clearance rate in women below the age of 30 years than in women above 30 years because of competent cell medicated immune systems in younger patients [4]. Therefore, a positive HPV DNA test in women over 30 years old is significant and should be followed up closely as it has a higher probability of inducing neoplastic changes [5]. This informed the choice of the study population in this study. The above discussion has shown that both the Pap smear and HR-HPV tests are not perfect. Would combining the two screening tests add any value in informing patient management strategies?

According to the American Society for Colposcopy and Cervical Pathology (ASCCP), HR-HPV and LBC co-testing offers an opportunity to increase screening intervals in women with NILM and HPV negative results from 3 years (when using LBC or HPV testing alone) to 5 years (with HPV/LBC co-testing) [6]. This may reduce the number of clinical consultations and therefore financial/time savings for patient and health funders. Fig. 1 below shows the ASCCP recommendations.

It is universally accepted that HR-HPV is a pre-requisite for cervical neoplasia [3]. However, not all HPV infected cells exhibit cellular atypia detectable on a Pap smear. Therefore, an NILM result does not exclude the presence of a current lesion or the possibility of a future cervical lesion. This dilemma can be cleared by co-testing with HR-HPV and LBC [7]. A positive high risk HPV result in women older than 30 years confers a higher risk of a future cervical lesion [7]. Therefore, HPV and LBC co-testing has a value of predicting possible cervical lesions in the NILM/HPV+ group. This helps to identify patients who need closer follow up for cervical lesions. The LBC/HR-HPV co-testing algorithm is shown in Fig. 2 below.

The Atypical Squamous Cells of Undetermined Significance (ASCUS) is a borderline diagnosis issued by pathologists if they are uncertain of the true nature of the lesion [6]. The differential diagnosis includes an exuberant reactive lesion and a true neoplastic lesion [6]. ASCCP recommends follow up by either repeat testing with a Pap smear after 6 and 12 months or triaging with high HPV testing to determine the nature of the lesion (reactive or neoplastic) [6]. Knowing the nature of the lesion helps clinicians to decide on the appropriate management strategy. Wilbur et al regarded the later as a more cost effective method than the former [5]. This is because co-testing allows immediate resolution of a borderline ASCUS result after conduction of two tests (baseline LBC and HPV testing) rather than three Pap smears (baseline, after 6 months and 12 months) [7]. In addition, co-testing enables instant resolution of the uncertainty which is important for the emotional well being of the patients.

The motivation for this study was that despite many health centres using the co-testing approach in the world, there is paucity of information regarding the clinical value of LBC/HR-HPV co-testing compared to screening using either HR-HPV or LBC individual tests. This study, therefore, aimed at assessing the clinical value of LBC/HR-HPV DNA co-testing in terms of increasing screening intervals from 3 to 5 years (HPV-/NILM group), predicting possible future cervical lesion (HPV+/NILM group) and resolution of uncertainty associated with borderline ASCUS LBC results in women ≥ 30 years.

	ACOG[17]	ASCCP[18]	USPSTF[19]
Pap only	Every 3 years	Every 3 years	Every 3 years
Pap-HPV cotest	Every 5 years, age 30–65	Every 5 years, age 30–65	Every 5 years, age 30–65
High-risk HPV only	Every 3 years, age > 25	Every 3 years, age > 25	Every 5 years, age 30–65

ACOG = American College of Obstetricians and Gynecologists; ASCCP = American Society for Colposcopy and Cervical Pathology; HPV = human papillomavirus; USPSTF = US Preventive Services Task Force

Fig. 1. American Society of Colposcopy and Cervical Pathology recommendations; adopted from Wilbur et al [5]

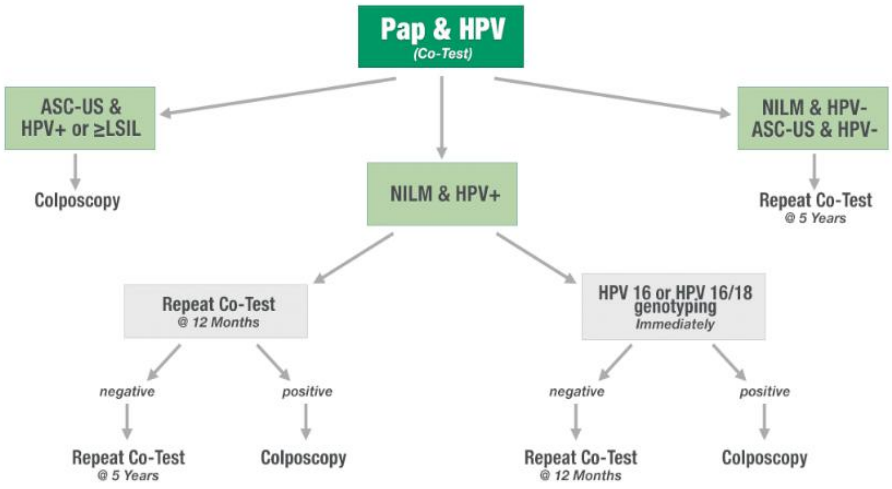

Fig. 2. An algorithm for LBC and HPV co-testing; Adopted from Wilbur et al [5]

Abbreviations- Pap: Papanicolaou smear cytology, HPV+: Human Papillomavirus positive test, HPV-: Human Papillomavirus negative test, NILM: Negative for Intraepithelial Lesion or Malignancy (NILM), ASCUS: Atypical Squamous Cells of Undetermined Significance, LSIL: Low Grade Squamous Intraepithelial Lesion

2. MATERIALS AND METHODS

2.1 Study Design

Cross-sectional descriptive study from February 2020 to November 2020.

2.2 Study Sites

Cimas Medical Laboratories**,** Harare, Zimbabwe.

2.3 Study Population

Women who came for cervical cancer screening at Cimas Healthcare Clinics.

2.4 Study Entry Criteria

Only women ≥ 30 years old with no prior history of cervical precancerous and cancerous lesions were enrolled in this study.

2.5 Sampling Method

Consecutive sampling method.

2.6 Sample Size

A total of 542 women who fulfilled selection criteria were recruited in this study.

2.7 Study Objective

To assess the clinical value of LBC/HR-HPV co-testing in terms of increasing screening intervals (in the HPV-/NILM group), predicting possible future cervical lesion (in the HPV+/NILM group) and resolution of the uncertainty associated with borderline ASCUS LBC results in women ≥ 30 years.

2.8 Sample Processing

The LBC and HR-HPV testing was conducted parallel on the samples.

2.8.1 Liquid based cytology smears

The samples were collected using a Cervex brush (Rovers Medical Devices – 5347 KV, Netherlands) and preserved in a Preserv Cyt solution (Hologic Inc – Marlborough, MA 01752 USA)[8-9]. A Thin Prep 2000 machine (Hologic Inc – Marlborough, MA 01752, USA) was used to deposit a monolayer of cells on to a Thin Prep charged microscopy slide (Hologic Inc – Marlborough, MA 01752 USA) according to the manufacturer's specifications [10]. The LBC slides were stained using the Papanicolaou stain.

2.8.2 LBC slides interpretation

The slides were reported using the 2014 Bethesda System of reporting cervical smears [6]. The LBC smears were evaluated by the principal investigator, a Clinical cytologist (MSc, Clinical Cytology) and a Pathologist (MMED, Anatomic Pathology) for the presence or

absence of epithelial abnormality. Discrepant findings were referred to a third person, a pathologist (MMED Anatomic Pathology). The third pathologist was blinded of the results of the first two reviewers. All interpreters were blinded of the HR-HPV test results.

2.8.3 HR-HPV DNA testing

HR-HPV DNA testing was done using the Cepheid Xpert HPV qualitative test (CE IVD-Sunnyvale, CA 94089 USA) according to the manufacturer's specifications [11]. The Xpert qualitative test detects 14 high risk HPV types which were reported as HPV 16, HPV 18/45 and other HR HPV (31,33,35,39,51,52,56,58,59,66 and 68) according to the kit manufacturer's specifications [11].

2.9 Data Management

Patients eligible for the study were assigned a unique study number and the following data was captured: age, date of last menstrual period and any clinical symptoms noted during clinical examination. LBC results, HR-HPV results and all prior data were stored in an IBM SPSS software version 21. Information stored in soft copies was protected from access from unauthorized persons by a password which was changed periodically. The data was analyzed used the IBM SPSS software version 21. Descriptive statistics were presented as proportions, tables and charts.

3. RESULTS AND DISCUSSION

A total of 551 paired samples were collected during the study period. Nine (1.6%) were excluded from the analysis because they had invalid HR-HPV results.

3.1 Age Characteristics

A total of 542 women of ages' ≥ 30 years had their variables analyzed in this study. Their age characteristics are summarized in Table 1 below:

3.2 HR-HPV and LBC Testing Results

3.2.1 HR-HPV results

Of the 542 specimens tested for HR-HPV DNA, 423 (78%) were negative and 119 (22%) were positive.

3.2.2 LBC results

Of the 542 LBC specimens evaluated for epithelial abnormality, 520 (96.0%) had NILM results. The remainder had: ASCUS (n=14, 2.5%), LSIL (n=5, 0.9 %) and HSIL (n=3, 0.6 %) diagnosis.

3.3 HR-HPV and LBC Findings for Each Age Group

The mean ages of the HR-HPV positive and HR-HPV negative were comparable (40.1 years vs. 38.9 years, respectively; p=0.412). LBC abnormalities were significantly higher in women >37years (median age) than women ≤37 years (18.2% [4/22] vs. 81.2% [18/22], respectively; *p*=0.002).

3.4 HR-HPV and LBC Result Combinations

The HR-HPV and LBC co-testing result combinations of the 542 specimens are illustrated in Fig. 3 below.

3.5 The Clinical Value of HR-HPV and LBC Co-Testing

The value is summarised in Fig. 3 below.

3.6 Discussion

The introduction of Pap smears in 1949 led to an enomous decrease in the incidence of cervical cancer in developing [2]. However, Pap smears have low sensitivity due to sampling and interpretation errors [2]. HPV DNA testing on the other hand, has a low specificity as a positive result has a poor correllation with clinical disease [7]. This shows that both screening tests are not perfect. Co-testing with LBC cytology and HPV DNA testing is the approach with enormous potential to improve cervical cancer screening in Zimbabwe.

In our study, co-testing had a value of extending the screening interval from 3 to 5 years in 418 (77.1 %) (NILM/HPV- patients), predicting possible future lesions in 102 (18.8%)(NILM/HPV- patients), confirmation of a neoplastic lesion from boarderline ASCUS lesions in 9 (1.7%) (ASCUS/HPV+ patients) and confirmation of a non neoplastic lesion from boarderline ASCUS lesions in 5 (0.9%) (ASCUS/HPV- patients).The major strength of this study is the large sample size which produced reliable conclusions.

Table 1. Age characteristics of study participants

Variable	Years
Mean age	39.4
Standard deviation	8.6
Median age	37
Age range	30-83
Peak age group	30-40

Table 2. HR-HPV and LBC findings for each age group

Age group	LBC results				HR-HPV results	
	NILM	ASCUS	LSIL	HSIL	Positive	Negative
31-40	251	2	2	0	33	222
41-50	109	3	1	0	48	65
51-60	72	4	0	1	21	56
61-70	58	2	1	2	9	54
71-80	23	3	1	0	7	20
81-90	7	0	0	0	1	6
Total	520	14	5	3	119	423

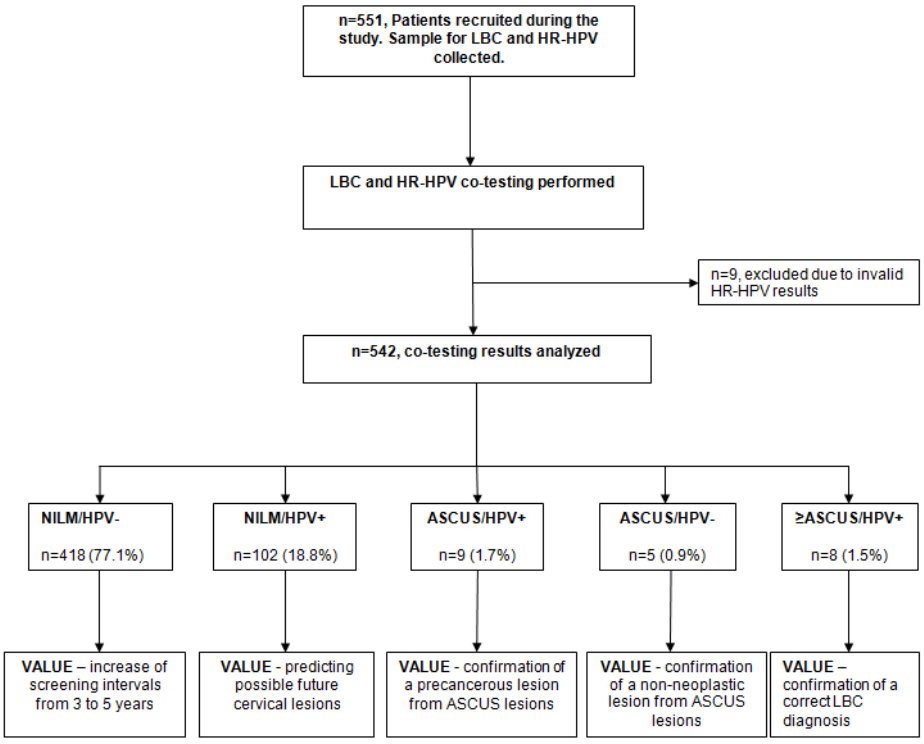

Fig. 3. HR-HPV and LBC result combinations and the value of LBC & HR-HPV co-testing

The detection of LBC abnormalities in this study increased with age as it was noted that patients below the median age (37 years) were less likely to have cervical lesions compared to patients above the median age, p=0.002. This is because it takes about eight years to several decades for cervical lesions to develop after an acute HPV infection [3]. In addition, the older patients are more likely to have acquired more somatic mutations compared to the younger patients [7]. The somatic mutations may affect the body's ability to repair damaged DNA as well as

performing apoptosis of the mutated cells [3]. It is documented that younger women have more competent cell mediated immunity that enables them to eliminate HPV infection [3]. However, in this study, the mean age of patients with or without an HPV infection were comparable; p=0.412. This may partly be explained by the fact that none of our enrolled patients was below 30 years where HPV clearance rates are higher.

Accoding to the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines for cervical cancer screening, patients with NILM/HPV- co-testing results are recommended from a follow up after 5 years [12]. This is an extension from the usual 3 year follow up period if either a Pap smear or HPV testing is done alone [12]. In this study, about 77% of the patients benefited from this extension if ASCCP guidelines were adhered to. The longer screening interval translates to fewer clinical consultations, decongestion of cervical cancer screening centres and increased accessibility to cervical cancer screening services to more patients. In addition, scarce cervical cancer screening resources in Zimbabwe could be chanelled to other unscreened populations. In support of this ASCCP recommendations, Cage et al reported that a negative HPV test reasures against future possible risk of cervical neoplasia [13].

It is universally accepted that HR HPV is a pre-requisite for cervical neoplasia [4]. However, not all HPV infected cells exhibit cellular atypia detectable on a Pap smear. Therefore, an NILM result cannot exclude the pressence of a current lesion or the possibilty of a future cervical lesion. This dilema can be cleared by co-testing [6]. A positive HR HPV result in women older than 30 years confers a higher risk for a future cervical lesions [5]. In this tudy 18.8% fell into that category and reqiure closer follow up with another co-testing after 12 months [5]. This approach results in closer follow up of such patients to prevent progression of the undetected or future lesions to more serious lesions [7]. The rate of NILM/HPV+ discrepant results in this study was 18.8% which is higher than the 4.1% recorded by Cormier at al in USA [14]. The difference could be due to higher prevalence of HPV infections and higher likelihood of HIV related immunosupression in our population compared to the USA population.

The Bethesda system of reporting cervical cytology recommends HR-HPV testing to triage patients with borderline ASCUS results [9]. Wilbur et al regarded this as a cost effective method compared to repeat cytology at 6 and 12 months recommended by ASCCP [5,6].Triaging with HR HPV enables determination of the nature of the lesion (neoplastic vs. non neoplastic lesion) from the same sample. In this study 1.7% and 0.9% of ASCUS results were confirmed as neoplastic and non neoplastic lesions respectively by triaging with HPV. Therefore co-testing in this case helped clinicians to make correct patient management decisions.

The major limitation in this study occurred in the ASCUS/HPV- category. In this study, we interpreted this finding as an non neoplasic or exuberent reactive lesion, however, this may have been due to a false negative HPV result. Catteau et al demonstrated that such false negative results may be due to low volumes of the Preserv Cyt solution [15]. Quiroga –Garza et al also reported that the ASCUS/HPV- discrepant finding could be due to rare HPV subtypes such as HPV 90 which are not available on current commercial kits [16].

4. CONCLUSION

The co-testing approach had clinical value of increasing screening intervals from 3 to 5 years in 77.1%, predicting possible future cervical lesion in 18.8%, confirmation of a precancerous lesion from ASCUS lesions in 1.7% and the confirmation of a non-neoplastic lesion from ASCUS lesions in 0.9% of all co-tested samples.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was obtained from the Joint Research Ethical Committee of University of Zimbabwe and Parirenyatwa Hospital (JREC), certificate number: JREC 3/2020. Permission was also granted by Cimas Medical Laboratories. During the study, strict patient confidentiality was observed. Cervical sample collection is a safe procedure. However, minor complications such as mild bleeding may be encountered in patients with cervicitis. Such spotting is usually self limiting and usually ends on its own in a few hours. Patients who received positive results were referred to gynecologists within the group for colposcopy and treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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