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UTILITY OF HIGH-RISK HPV (HR-HPV) AND LIQUID BASED CYTOLOGY (LBC) FOR CERVICAL CANCER SCREENING OF UNSCREENED WOMEN OVER 29 YEARS OLD IN HARARE, ZIMBABWE
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UTILITY OF HIGH-RISK HPV (HR-HPV) AND LIQUID BASED CYTOLOGY (LBC) FOR CERVICAL CANCER SCREENING OF UNSCREENED WOMEN OVER 29 YEARS OLD IN HARARE, ZIMBABWE

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ABSTRACT

Background: **Approximately 50% of cervical cancer cases are diagnosed in women who have not been screened previously. It has been observed in some centres that co-testing with LBC and HR-HPV identifies those with current precancerous lesions and those with an active HR-HPV for management. The procedure has not been examined in our setting so as to enable consideration for use in the diagnostic process.**

Objective: **To determine LBC and HR-HPV findings in women over 29 years old who had no previous screening for cervical cancer at Cimas MEDLABS.**

Design: **Cross sectional descriptive study**

Materials and Methods: **Women with no prior history of cervical screening were recruited into this study. A Thin Prep 2000 machine was used process the LBC specimens. The 2014 Bethesda System was used to report the smears. HR-HPV DNA testing was done using the Cepheid Xpert HPV qualitative test.**

Results: **There were 1446 co-tested specimens; the mean (SD) age of study participants was 38.7 (8.3) years and the range was 30-79 years. Tests results showed the following: NILM/HPV- (n=1149, 79.5%), NILM/HPV+ (n=265, 18.3%), ≥ ASCUS/HPV+ (n=23, 1.6%), ASCUS/HPV- (n=9, 0.6%). The ≥ ASCUS lesions were distributed as follows: ASCUS = 25, LSIL = 6 and HSIL = 1. The co-testing approach**

identified 297 (20.5%) women with a higher risk of developing cervical cancer (≥ ASCUS and HPV+ patients) for follow up according to established protocols.
***Conclusion*: Approximately a fifth (20.5%) of unscreened women had either a precancerous lesion or an active HR-HPV infection and required follow.**

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 (2). 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).

Indeed the incidence of cervical cancer in developed countries is showing a downward trend over the past seven decades since 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 (1). This is attributed to low utilisation of methods of screening known to improve population cervical cancer screening programs effectiveness. Furthermore, this compromises accessibility to cervical cancer screening services in Zimbabwe resulting in millions of women not being screened.

Subramaniam et al reported that approximately 50% of cervical cancer cases are diagnosed in patients who have never been screened (4). Andrae et al also reported that unscreened women have greater odds (2.5) of developing cervical cancer compared to women who are screened regularly (5). The Pap smear detects precancerous lesions which are treatable (3). Liquid based cytology (LBC) is a newer technique for the preparation of cervical smears which permits HR- HPV DNA testing on the same sample (6)

The HPV infection is a pre-requisite for cervical neoplasia as it is implicated in the causation of 99.7% of genital tract cancers (7). Therefore, HR-HPV testing enables stratification of women according to the risk of developing cervical cancer. The advantages for HR-HPV testing are high reproducibility, high sensitivity, and high negative predictive value (NPV) (8). However, the major limitations for HR-HPV DNA testing are; low positive predictive value (PPV), low specificity and poor correlation with clinical disease (9). A positive HPV result does not always signify the presence of a treatable cervical lesion. Thus, HR-HPV results should be interpreted with caution (9).

The HR-HPV has higher clearance rate in women < 30 years old than in women ≥ 30 years old because of competent cell medicated immune systems in younger patients (9). Therefore, a positive HPV DNA test in women ≥ 30 years old is significant and should be followed up closely as it has a higher probability of inducing neoplastic changes (6). This explains the choice of the study population in this study. This study sought to screen an unscreened population of women > 29 to identify those with current cervical lesions and those with an active HR-HPV infection for closer follow up.

MATERIALS AND METHODS

Study design: This study was a cross-sectional descriptive study from January 2017 to December 2019.

Study sites: Cimas Healthcare Clinics and Cimas MEDLABS. Cimas is a medical company in Zimbabwe that has clinics, laboratories, medical aid, pharmacies, ambulances and dialysis units. Cimas MEDLABS is one of the few sites offering both HR- HPV and LBC tests in Zimbabwe.

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

Study entry criteria: Only women over 29 years old who had no previous screening for cervical cancer were enrolled in this study.

Sampling method: Consecutive sampling method was used in this study.

Sample size: A total of 1446 women who fulfilled selection criteria were recruited in this study

Study objective: To determine LBC and HR-HPV findings in women >29 years old who have never been screened for cervical cancer at Cimas Medical Laboratories.

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

Liquid Based Cytology smears

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). The LBC slides were stained using the Papanicolaou stain.

LBC slides interpretation

The slides were reported using the 2014 Bethesda System of reporting cervical smears. 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 reviewers were blinded of the HR-HPV test result.

HR-HPV DNA testing: HR-HPV DNA testing was done using the Cepheid Xpert HPV qualitative test (CE IVD- Sunnyvale, CA 94089 USA). 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).

Data Management: Patients eligible for the study (women over 29 years old who had no previous screening) 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. After evaluation of the LBC samples, the LBC and 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.

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 MEDLABS. 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 for colposcopy and treatment.

RESULTS

A total of 4192 women were evaluated for cervical cancer at Cimas Medical Laboratories during the study period. Of these 1446 (34.5%) met the inclusion criteria of this study and were recruited.

Age characteristics of study participants.

The mean (SD) age of the patients from whom the specimens were collected was 38.7 (8.3) years and the range was 30-79 years. The median age was 36 years. Figure 2 below shows the age characteristics of the study participants with a peak in the age group 30-40 years.

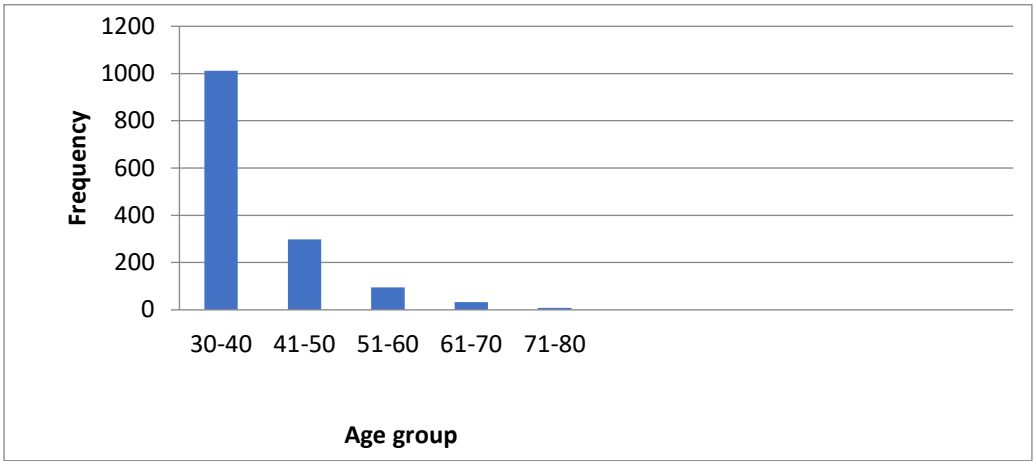

Figure 2: Age characteristics of the study participants.

HR-HPV and LBC testing results

HR-HPV results: Of the 1446 specimens tested for HR-HPV DNA, 1158 (80%) were negative and 288 (20%) were positive.

LBC results: Of the 1446 LBC specimens evaluated for epithelial abnormality, 1 414 (97.8%) had NILM results. The remainder had ASCUS 25 (1.7 %), LSIL 6 (0.4 %) and HSIL 1 (0.1 %) diagnosis.

HR-HPV and LBC result combinations: The 1446 co-tested specimens had the following result combinations; NILM/HPV- (n=1149, 79.5%), NILM/HPV+ (n=265, 18.3%), ≥ ASCUS/HPV+ (n=23, 1.6%), ASCUS/HPV- (n=9, 0.6%). The ≥ ASCUS lesions were distributed as follows: ASCUS = 25, LSIL = 6 and HSIL = 1. The co-testing approach identified 297 (20.5%) women with a higher risk of developing cervical cancer (≥ ASCUS and HPV+ patients) for follow up according to established protocols.

DISCUSSION

Cervical cancer development is supposedly a multistep process that evolves from an acute HPV infection to progressively more serious precancerous lesions (CIN I to CIN III) (3). The Pap smear offers the possibility of detecting the earlier precancerous lesions for treatment before they become invasive (6). It therefore, follows that approximately 80% of invasive cervical cancers are diagnosed in patients who have never been screened before (4). This study sought to screen an unscreened population of women > 29 years to identify those with current cervical lesions and those with an active HR-HPV infection for closer follow up.

There were in this study, 1158 (80%) specimens were negative for HR-HPV and 288

(20%) were positive for HR-HPV. Of the 1146 samples evaluated for epithelial abnormality, 1 414 (97.8%) had NILM results. The remainder had ASCUS 25 (1.7 %), LSIL 6 (0.4 %) and HSIL 1 (0.1 %) LBC results. The proportion of patients that had abnormal LBC results in this study was lower than the one reported by Mandishora et al who recorded a higher rate of abnormal smears (30%). The higher rate of abnormal LBC in the later study can be explained by a higher proportion of HIV infected women enrolled in that study.

The HPV positivity in this study (20%) was comparable to a study done by Fitzpatrick in a rural population in Zimbabwe which reported an HPV positivity rate of 17% (10). This confirms that our study findings are generalizable to any part of the country. However, the HPV positivity rate in this study was lower than that recorded by Mandishora and Sharita et al which reported HPV positivity rates of 72% and 43% respectively (11,12). This difference can be explained by the enrollment of a higher proportion of HIV positive patients. Literature shows that HIV positive women are more likely to have an HPV infection, multiple HPV subtypes, more high-risk HPV subtypes and higher HPV viral load than to HIV negative patients (3).

This study's results showed that 18.3% women had the NILM/HPV+ result combination. This group had no current detectable lesion; however, because they are HR-HPV infected they have a higher risk of developing precancerous lesions. Therefore, this group requires a closer follow up so that future cervical lesions are detected early (13-14). The rate of NILM/HPV+ discrepant results (18.3%) in this study was higher than the rate of 4.1% recorded by Cormier at al in USA (15). The difference could be due to higher prevalence of HPV infections and higher likelihood of HIV related immunosuppression in our population compared to the USA population.

In this study, 9 (0.6%) specimens had ASCUS/HPV- result combinations. This can be caused by exuberant reactive lesions which were incorrectly classified as ASCUS lesions (6). However, this may have been caused by 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 (16). 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 (17).

CONCLUSION

Approximately a fifth of unscreened women aged over 29 years old either had precancerous lesion or an active HR-HPV infection and required follow. We recommend that LBC/HR-HPV co-testing should be used to screen women who have no previous screening as it enables identifying women with both precancerous lesions and those with clinically significant HR-HPV infections so that appropriate follow up could be taken.

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