Evaluation of a Low-Cost Liquid-Based Pap Test in Rural El Salvador: A Split Sample Study

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ABSTRACT

BACKGROUND: Liquid-based cytology facilitates morphologic interpretation and enables ancillary molecular testing of Pap tests when compared to the conventional Pap. Increased cost over the conventional method have precluded the introduction of liquid-based methods in developing countries. We sought to test the diagnostic efficacy of a low-cost, liquid based Pap that could be implemented in low-resource settings.

METHODS: A prospective, split-sample Pap study was performed in 595 women attending a cervical cancer screening clinic in rural El Salvador. Collected cervical samples were used to make a conventional Pap while residual material was used to make the liquid-based sample using the ClearPrep® method. Selected samples were tested from the residual sample of the liquid-based collection for the presence of high-risk Human papillomaviruses (hr-HPV).

RESULTS: Excellent agreement was found between cytology results for the two methods, with overall kappa of 0.860. Five hundred seventy of 595 patients were interpreted with the same exact diagnosis between the two methods (95.8% agreement). There were comparable number of unsatisfactory cases however ClearPrep significantly increased interpretation of LSILs and HSILs and fewer diagnoses of ASCUS. Hr-HPV was identified in all cases of HSIL, AIS, and cancer as well as in 78% of LSIL.

CONCLUSIONS: The low-cost ClearPrep Pap test demonstrated superior detection of SIL when compared to the conventional Pap smear and demonstrated the potential for ancillary molecular testing. The test appears a viable option for implementation in low-resource settings.

INTRODUCTION

Cervical cancer screening has substantially reduced the incidence and mortality of cervical cancer in the United States of America and Western Europe [1]. Despite the introduction of the Pap into many developing countries, similar reductions in cervical cancer have not been achieved[2]. In Mexico, increased penetration of the Pap test examined in several provinces failed to coincide with an overall decrease in cervical cancer mortality within the same period [3]. Possible explanations for the failure of the Pap test in countries with limited resources have ranged from inadequately trained screeners, poorly prepared tests, or inadequate follow-up of patients with positive tests[2, 4]. Some carefully conducted collaborative studies between the US and some Central American countries have performed extensive quality assurance on screeners in these countries. The findings in these studies show equivalence in diagnostic competence between the foreign and US screeners [5], however studies evaluating the quality of Paps prepared in these countries have reported numerous factors that limit the quality of the Pap staked in these Countries, with poor fixation virtually always being one of the most common[2, 6, 7]. Improvement in the optical quality of the actual Pap test was an initial justification for liquid based cytology in the United States and a possible reason for the success of liquid based cytology in this Country. Numerous studies comparing conventional and liquid based Pap argue to an improvement in the sensitivity using the liquid based test[8-10]. In addition, the residual sample from liquid based cytology methods is an excellent medium for the performance of ancillary molecular studies that have been shown to enhance the management of patients screened with the Pap[11]. The introduction of liquid based cytology to developing countries has been historically precluded by the significant increase in cost of these tests. With the emergence of a low cost HPV test that is targeted for developing countries and can be performed out of liquid-based fluids[12], we sought to prospectively establish the diagnostic performance of a low cost liquid based cytology in El Salvador to explore how the use of such a medium could assist in cervical cancer screening in that country.

METHODS

This is a prospective study of women seeking screening in rural El Salvador. The study was conducted as a part of a cervical cancer screening prevention program directed under the auspices of Basic Health International, a non-profit organization dedicate to the eradication of cervical cancer in Central America. The study was approved by the Internal Review Board of the National Ministry of Health of El Salvador, by that of New York University and of the University of Southern California.

Six hundred one women ages 30 through 49 were accepted participation in this study. The study consisted of adding a Pap test to the standard screening method offered in that visit, direct visual inspection with acetic acid (VIA). After verbal and written informed consent, each woman enrolled in the study received a conventional Pap test performed in the standard fashion using a spatula and endocervical brush. Following the standard smear onto a glass slide for the conventional Pap, the devices were rinsed in a ClearPrep® liquid vial in a fashion previously published using other liquid based methods (split-sample method)[13]. Both conventional and ClearPrep samples were given deidentified study numbers that kept the identity of each patient masked from the participating cytotechnologists and cytopathologists until the end of Pap

reporting. Conventional Pap and ClearPrep slides were screened blindly and independently by 4 cytopathologists with a consensus diagnosis reported in the results. The Bethesda system terminology was used for reporting cervical cytology results[14]. Each patient was subsequently screened with VIA and managed according to the results of that test. Once all Pap diagnoses were final, the data for each patient was unmasked and the results compared. Following adjudication of both conventional and ClearPrep Paps, patients with a positive Pap test that did not receive appropriate management as a result of VIA screening received an appointment for additional management as per her most abnormal Pap result.

Testing for high-risk Human papillomavirus was performed on selected samples. All cases with an abnormal Bethesda System diagnosis (ASC, AGC, LSIL, HSIL, and cancer) were tested for high-risk HPV, if sufficient sample was available. In addition, ten random samples with a NILM diagnosis were also tested for high-risk HPV. The residual spun-down, resuspended sample was retrieved and a single drop placed into the Genfind DNA extraction kit provided with the Cervista hr-HPV test kit.[15] The assay was then performed as indicated in the package insert.

Statistical analysis was calculated with paired Chi-Square test between conventional and ClearPrep Pap methods. Kappa-measure was used to evaluate level of agreement between the two methods. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Result:

A total of 601women were examined during the study. All women accepted participation in the study. Specimen from 6 participants were lost during the process leaving 595 samples for comparison. Cytologic diagnosis for both conventional and ClearPrep preparations are summarized in Table 1. Abnormal Pap smears were detected in 19 (3.19%) patients in the ClearPrep group as compared to 18 (3.03%) patients in the conventional group (p=0.994). No significant difference was found in the proportion of unsatisfactory samples between conventional smear group, 8 (1.34%) cases and the ClearPrep group, 7 (1.17%) cases (p=0.998). Lower number of ASCUS was diagnosed by the ClearPrep method (1 case, 0.17%) than that with conventional method (8 cases, 1.34%) with significant statistical difference ($p<0.001$). The 8 cases of ASCUS interpreted by conventional Pap include 4 cases of LSILs, 1 case of HSIL, 1 case of ASCUS-H and 2 cases of NILM, suggesting a higher discrimination of the ClearPrep method over the conventional Pap in identifying abnormalities. There was also significant difference in ASC-H diagnosis: 1 case (0.17%) with coventional as compared to 2 cases (0.34%) with ClearPrep method (p=0.03). The 2 cases of ASC-H detected by ClearPrep include 1 case of ASC-H and 1 case of ASCUS in the conventional Pap group. Significantly increased number of LSILs and HSILs were found in ClearPrep as compared to conventional Pap (9 vs 4 cases and 5 vs 3 cases, $p<0.001$ respectively). Interestingly, the 4 LSILs detected by conventional Pap were all detected by ClearPrep method, with the remaining 5 LSILs being 4 ASCUS and 1 NILM by conventional Pap. Of the 5 HSILs detected by ClearPrep, 2 were detected as HSIL by conventional Pap while the other 3 HSILs were interpreted as AEC, ASCUS and NILM respectively. Of all the 3 HSIL detected by conventional Pap, 2 cases were also intertreted as

HSIL and 1 case as SCC by ClearPrep Pap. These data suggest that ClearPrep was more definitive in detecting LSILs and HSILs than conventional Pap and tends to have a low rate of ASCUS.

There were 2 cases of AECs detected by conventional Pap, of which 1 case was detected as AIS and the other one was interpreted as HSIL by ClearPrep, suggesting that ClearPrep may be more definitive in cervical glandular lesions and tends to lower interpretation for AEC.

Taking into account each of the 595 cases in the study, there was excellent agreement between the conventional and ClearPrep Pap methods in detecting cervical intraepithelial lesions as shown in Table 2. There was good correlation between the two methods as interpreted by Kappa measure: 0.86 (95.8% CI: 0.74-0.98). These data suggest that a split-sample liquid-based ClearPrep Pap is as good a screening test as the conventional test and can be used as an alternative in screening for cervical intraepithelial lesions. Moreover, as the cost of ClearPrep is dramatically reduced as compared to the currently used liquid-based Pap tests, ClearPrep may play an important role in screening cervical intraepithelial lesions in developing countries where resources in preventive medicine are limited.

Limited high-risk HPV testing of the residual sample generated from the ClearPrep sample reveled that 5 of 5 (100%) HSIL, and 7 of 9 (78%) LSIL samples tested positive for high-risk HPV. The one case of cancer and one case of AIS both tested positive, while the sole ASC-US case tested negative for high-risk HPV. Of the ten sample with a diagosis of NILM, only one sample (10%) was positive for high-risk HPV.

DISCUSSION

We have evaluated the diagnostic performance of a low-cost, liquid based Pap test in rural El Salvador. Most of the currently used liquid-based Pap tests are performed in North America and Western Europe take advantage of a fluid transport medium to preserve cells and to eliminate debris and distribute a more representative portion of cells on a slide in a uniform and even layer of cells. These liquid-based Pap tests allow a clearer, easier slide to read, and minimizes obscuring blood, mucus, and non-diagnostic debris[16-21]. The major disadvantage of the currently used liquid based Pap tests is the cost incurred mainly by the sophisticated and expensive machines utilized in making the slides, limiting its use in small laboratories of rural areas in developing countries.[19-23]

The advantages of the liquid-based test utilized in this study was the better cell preservation that appeared to improve visual clarity, facilitating more definitive Pap interpretations. This was suggested by the reduced ASCUS rate with a concomitant increase in SIL diagnoses. Increased SIL diagnoses could also be due to a more representative sampling in the liquid-based method as compared to the conventional Pap. With the conventional Pap, as much as 80 percent of every cell sample is discarded when the swab or spatula used in collecting the sample is disposed. The small fraction of a cell sample that's smeared onto a glass slide for microscopic analysis may not include cell abnormalities that are present in the discarded cells[22-24]. Finally, blood, mucus, and inflammation frequently present in the conventional smear often times make it difficult or impossible to accurately analyze some of the slides.

Although the conventional Pap smear in this study was made first, in this split-sample study, slightly fewer unsatisfactory smears were seen with the latter preparation. The cell re-suspension process in ClearPrep enables a more representative sample portion, reduces obscuring blood, mucus, and nondiagnostic debris. It has also the advantage of being able to produce more than one diagnostic slide as well as make material available for ancillary test sent-outs[16-24]. In fact, if a split-sample liquid-based ClearPrep is comparable to or even better than the conventional Pap test, the direct-to-vial Pap sampling would be a more adequate and better alternative to the conventional Pap.

We also tested selected samples for the presence of high-risk Human Papillomaviruses. Positive reactivity of all HSIL, cancer and AIS tested with only one positive test among the NILM population in our study suggests that the fixative used with the ClearPrep method is an adequate transport medium for the detection of high-risk HPV. This is of particular importance as a low-cost HPV test is currently in the initial phases of implementation across several developing countries and at least some strategies include HPV testing followed by a reflex Pap test. This strategy relies on the availability of a liquid based sample.

The reduced of the ClearPrep Pap over the currently used ThinPrep or SurePath Pap tests makes using such a strategy feasible. Currently suggested cost of other liquid based methods averages over $6 US, while the ClearPrep method costs significantly less. In addition, the ClearPrep Pap test employs a centrifugation process instead of a complicated and expensive machine in slide processing. The markedly reduced cost of this test would facilitate more advanced, sophisticated screening of one of the most deadly but preventable malignancies affecting women in developing countries.

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