Molecular-Based HPV Screening in Low Resource Countries

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ABSTRACT

Study Objective: Cervical cancer rates in low- and middle-income countries (LMICs) are 3 times higher than those in developed countries. In Honduras, cervical cancer is the most common cancer affecting women, accounting for approximately 417 deaths annually in a population of less than 8 million. Nearly all cervical cancers are caused by human papillomavirus (HPV) infection and implementing a low-cost, rapid, near patient HPV screen would greatly improve cervical cancer outcomes. In collaboration with La Liga Contra el Cancer in Honduras we have adapted HPV tests from QuanDx® for both high and low-risk HPV to provide rapid results that can assist in screening. We sought to provide insight on the prevalence of HPV infection in the population by identifying the high-risk and low-risk genotypes present.

Methods: We used the MeltPro High Risk and Low Risk HPV Genotyping assays (QuanDx/Zeesan Biotech, San Jose, CA) to detect 14 high-risk and 14 low-risk HPV subtypes through melt curve analysis. DNA was extracted by an alkaline lysis boiling of cervical swabs collected from 111 women in remote La Mosquitia, Honduras. Once isolated, DNA samples were added to the lyophilized MeltPro reagents, amplified on the SLAN-96 real time PCR instrument (QuanDx/Zeesan Biotech, San Jose, CA), and analyzed for HPV genotypes. DNA samples that failed to yield a melt-curve were re-extracted with the Lab-Aid® 824 and re-analyzed. The results were reported to physicians in Honduras to provide follow up care for women at risk for cervical cancer from HPV infection.

Results: Approximately 35% of the population examined had an HPV infection; 27 samples tested positive for high-risk HPV strains and 18 samples were positive for low-risk HPV strains. The most prevalent high-risk HPV genotypes were HPV-52 and HPV-16. The most prevalent low-risk HPV genotype was HPV-72, which represented 18% of the low-risk infections. Re-extraction of DNA samples with the Lab-Aid® 824 increased the ability to detect HPV and resulted in the discovery of 9 additional high-risk HPV infections and 3 additional low-risk HPV infections reducing the "invalid" rate from 10-13% to 0%.

Conclusions: HPV infections are prevalent in the La Mosquitia region of Honduras and the genotype distribution differs from that of developed countries and our previous data of less isolated communities in Honduras. In the future, the implementation of a cervical screening program utilizing molecular HPV testing would greatly improve the identification of at risk individuals and could help reduce the cervical cancer rate in Honduras.

BACKGROUND

Human Papillomavirus and Cervical Cancer: Human papillomavirus (HPV) is the most common sexually transmitted infection and is the primary etiological agent in the development of nearly all cases of cervical cancer. HPV is often divided into two distinct subtypes: high-risk HPV (oncogenic) and low-risk HPV (non-oncogenic). Oncogenic subtypes of HPV can cause the malignant transformation of cervical epithelial cells through interactions between viral proteins E6 and E7 and the tumor suppressor gene p53 and cell cycle regulator pRB.

Molecular HPV Testing: Molecular HPV testing allows more women to be screened with a more sensitive assay when compared to traditional cytology. Due to the high sensitivity of molecular HPV testing, co-testing (the combination of HPV testing and cytology) has become increasingly prevalent in developed countries such as the United States. In LMICs like Honduras, molecular HPV testing as a primary screen for cervical cancer has the ability to identify women at high risk of cervical cancer while remaining low-cost and reducing the burden on a limited number of trained pathologists in the country.

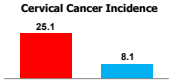

Figure 1: Cervical cancer incidence in Honduras and the United States. Numbers represent the crude annual incidence per 100,000 women. (Adapted from HPV Centre).

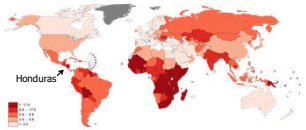

Figure 2: Map showing cervical cancer mortality rates per 100,000 women (World Health Organization, 2012)

Cervical Cancer in Honduras: Cervical cancer is the number one cause of cancer death in women in Honduras and is responsible for an estimated total of 991 new cases per year. Current cervical cancer screening in Honduras is dependent on a small number of pathologists analyzing Pap smear for abnormal cytology. This screening algorithm becomes problematic when long turn around times coupled with isolated populations delay or prevent early interventions. Previous collaborations between La Liga Contra el Cancer and the Norris Cotton Cancer Center (NCCC) have explored the efficacy of molecular based HPV testing as a means of cervical cancer screening in both urban and rural communities of Honduras.

Objectives:

- Identify high-risk HPV and low-risk HPV genotypes within the tested population.
- Determine overall HPV prevalence in the community

Figures 3: Pictures taken in the region of La Mosquitia, Honduras throughout the duration of the clinic.

METHODS

Figure 4: Representative examples of crude lysates for HPV genotyping

Crude DNA extraction: 111 cervical swabs from a one day outreach clinic in La Mosquitia, Honduras were obtained by La Liga Contra El Cancer and sent to Dartmouth-Hitchcock Medical Center. Swabs were placed in 0.4mL lysis buffer (50 mM NaOH, 0.2 mM EDTA), incubated at 95°C for 8 minutes, spun, and neutralized with 0.2 mL Tris pH 8 to obtain a crude DNA lysate. This procedure represents both a low cost and amenable extraction procedure to near patient conditions in Honduras (Figure 4).

HPV Genotyping: 25 µl of lysate was added to lyophilized reagents for both the MeltPro HR and LR HPV Genotyping kits (QuanDx, San Jose, CA). Reactions were run on a ZSLAN 96S real time PCR instrument (St. Louis, MO). Individual high-risk and low-risk HPV genotypes were determined through multi-color melt curve analysis (Figure 5) in which every HPV subtype has a unique Tm (Tables 1 & 2).

Crude lysate cleanup: In order to remove potential PCR inhibitors from crude lysates, previously run "invalid" samples were re-extracted using the LabAid® 824 DNA extraction system and then re-run. Next, crude lysates were diluted and re-tested with the Low-risk HPV Genotyping assay.

Confirmation testing: Positive HPV-52 samples were also examined using a SYBR® Green LDT for HPV.

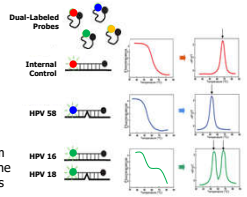

Figure 5: Probe based chemistry and melt curves (adapted from QuanDx®)

Channel	HR Genotype	Tm (°C)
ROX	HPV-31	49 ≤ Tm < 53°
	HPV-33	53 ≤ Tm < 58°
	HPV-16	59 ≤ Tm < 64°
	HPV-35	64 ≤ Tm < 68°
	HPV-68	68 ≤ Tm < 73°
	HPV-18	73 ≤ Tm < 77°
CY5	HPV-56	46 ≤ Tm < 52°
	HPV-52	52 ≤ Tm < 57°
	HPV-45	57 ≤ Tm < 63°
	HPV-39	63 ≤ Tm < 70°
FAM	HPV-59	44 ≤ Tm < 48°
	HPV-66	48 ≤ Tm < 54°
	HPV-58	54 ≤ Tm < 60°
	HPV-51	60 ≤ Tm < 74°
HEX	Internal Control	80 ≤ Tm < 70°

Table 1: High-risk HPV Tms.

Channel	LR Genotype	Tm (°C)
ROX	HPV-42	49 ≤ Tm < 54°
	HPV-69	54 ≤ Tm < 62°
	HPV-81	62 ≤ Tm < 66°
	HPV-43	66 ≤ Tm < 71°
	HPV-40	71 ≤ Tm < 77°
CY5	HPV-72	48 ≤ Tm < 53°
	HPV-44	53 ≤ Tm < 57°
	HPV-61	57 ≤ Tm < 63°
	HPV-54	63 ≤ Tm < 67°
	HPV-70	67 ≤ Tm < 73°
FAM	HPV-11	50 ≤ Tm < 56°
	HPV-6	56 ≤ Tm < 60°
	HPV-53	60 ≤ Tm < 66°
	HPV-26	66 ≤ Tm < 76°
HEX	Internal Control	80 ≤ Tm < 76°

Table 2: Low-risk HPV Tms.

RESULTS

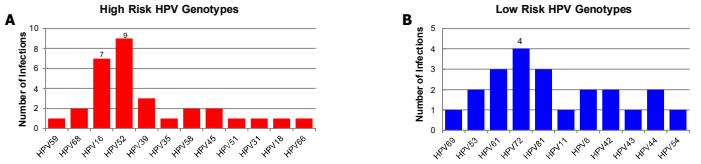

Figure 6A and B: Genotype distribution within the tested population for (A) high-risk and (B) low-risk HPV genotypes.

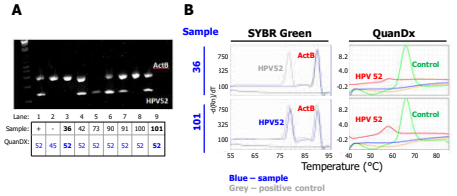

Figure 7: SYBR® Green QuanDx comparison. (A) Gel electrophoresis of samples showing the internal control (ActB) and HPV-52 L1 amplicon. (B) SYBR® Green and QuanDx® melt peaks for samples 36 and 101 shown.

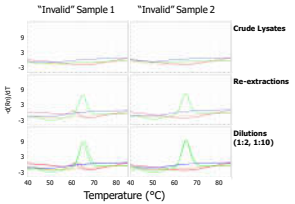

Figure 8: Crude lysate sample inhibition. Melt curve analysis for crude lysates, "re-extractions", and both 1:2 and 1:10 dilutions of crude lysates.

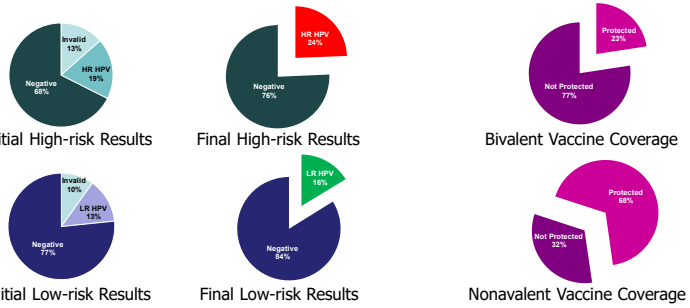

Figure 9: Comparison of initial results after DNA extraction utilizing boiling alkaline lysis method to final results after DNA re-extraction on Lab Aid ® 824 for high and low-risk HPV.

Figure 10: Summary of bivalent and nonavalent HPV vaccine coverage of identified high-risk genotypes.

CONCLUSIONS

- High-risk and low-risk genotype distribution in the tested population differs from that in more developed countries and previously tested communities in Honduras. Moreover current vaccine programs may not cover the most prevalent genotypes in isolated communities.
  - **High-risk HPV prevalence**
    - Tested population: HPV-52
    - Developed countries: HPV-16/18
  - **Low-risk HPV prevalence**
    - Tested population: HPV-72
    - Developed countries: HPV-6/11
- Dilution of crude lysates and robust lyophilized reagents offer improvements to HPV screening in difficult conditions.
- It is necessary to continue testing communities in Honduras for HPV genotype distribution in order to improve current cervical cancer screening programs and promote prevention through education and the use of appropriate vaccines.

ACKNOWLEDGEMENTS & REFERENCES

Acknowledgements: This work was supported by a Munck-Pfefferkorn Grant. In addition, the authors would like to thank all supportive Departments at DHMC, especially the Laboratory for Clinical Genomics and Advanced Technology and Department of Pathology and Laboratory Medicine.

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