Florida International University
FIU Digital Commons

FIU Electronic Theses and Dissertations | University Graduate School

6-28-2018

Cervical Infection with high risk Human Papillomavirus Anogenital Subtypes in Indigenous Women in Alta and Baja Vera Paz Guatemala

Anne Jeffries
Florida International Universty, ajeff004@fiu.edu

DOI: 10.25148/etd.FIDC006881
Follow this and additional works at: https://digitalcommons.fiu.edu/etd

 Part of the Community Health and Preventive Medicine Commons, International Public Health Commons, and the Women's Health Commons

Recommended Citation
Jeffries, Anne, "Cervical Infection with high risk Human Papillomavirus Anogenital Subtypes in Indigenous Women in Alta and Baja Vera Paz Guatemala" (2018). *FIU Electronic Theses and Dissertations*. 3754.
https://digitalcommons.fiu.edu/etd/3754

This work is brought to you for free and open access by the University Graduate School at FIU Digital Commons. It has been accepted for inclusion in FIU Electronic Theses and Dissertations by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

CERVICAL INFECTION WITH HIGH-RISK HUMAN PAPILLOMAVIRUS ANOGENITAL SUBTYPES IN INDIGENOUS WOMEN IN ALTA AND BAJA VERA PAZ, GUATEMALA

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PUBLIC HEALTH

by

Anne Jeffries

2018

To: Dean Tomás R Guilarte
Robert Stempel College of Public Health and Social Work

This dissertation, written by Anne Jeffries, and entitled Cervical Infection with High-Risk Human Papillomavirus Anogenital Subtypes in Indigenous Women in Alta and Baja Vera Paz, Guatemala, having been approved in respect to style and intellectual content, is referred for your judgment.

We have read this dissertation and recommend that it be approved.

Eric Fenkl

Purnima Madhivanan

Virginia McCoy

Consuelo Beck-Sague
Major Professor

Date of Defense: June 28, 2018

The dissertation of Anne Jeffries is approved.

Dean Tomás R Guilarte
Robert Stempel
College of Public Health and Social Work

Andres Gil
Vice President for Research and
Economic Development and
Dean of the University Graduate School

Florida International University, 2018

© Copyright 2018 by Anne Jeffries

All rights reserved.

DEDICATION

I dedicate this dissertation to my husband, Gordon, and children, Maggie and Curt, who put up with my searching.

ACKNOWLEDGMENTS

I would like to gratefully acknowledge the following persons, who contributed to this dissertation. First, Dr. Michael Dean, MD, Senior Investigator, Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, who shared his Guatemala study's HPV high risk subtype data for my dissertation. Second, I would like to recognize my colleagues in Partners in Surgery, United States, and the Guatemala affiliate (Compañeros en Cirugía, Antigua, Guatemala) who generously shared their data, and graciously accepted the results of a rigorous evaluation of the visual inspection with acetic acid (VIA) cervical cancer screening technique. The collaboration and selfless dedication to the work of outreach to the indigenous women of Alta and Baja Vera Paz of the nurses who participated in the VIA visits to rural communities is gratefully appreciated. Finally, and most importantly, I want to acknowledge the Promotoras de Salud (Health Promoters), who had the confidence of community women, and the women themselves, who walked to, and then waited patiently for hours for their examinations. Obviously, they were my most important collaborators. On behalf of my major professor and my dissertation committee, and with great humility and gratitude, I send to them a heartfelt ¡Maltiox chech alak! ¡Gracias!

I would like to thank my professors and especially my committee members for their support. I was not a traditional student, however, Dr. McCoy and Dr. Madhivanan always encouraged me on a rather steep learning curve. Dr. Fenkl stepped in when I needed to replace a committee member, and I will always be grateful for his commitment.

There is no way I could thank Dr. Consuelo Beck-Sague enough for her patience and mentoring. Her expertise, support, and guidance helped me accomplish a life-long educational goal.

"…inequities have powerfully sculpted not only the distribution of infectious diseases, but also the course of disease in those affected."

"We saw oppression. It looked, well, different from our comfortable lives in the university; and so we called it culture." —Paul Farmer, MD, PhD Infections and Inequalities

ABSTRACT OF THE DISSERTATION

CERVICAL INFECTION WITH HIGH-RISK HUMAN PAPILLOMAVIRUS ANOGENITAL SUBTYPES IN INDIGENOUS WOMEN IN ALTA AND BAJA VERA PAZ, GUATEMALA

by

Anne Jeffries

Florida International University, 2018

Miami, Florida

Professor Consuelo Beck-Sagué, Major Professor

Cervical cancer, caused by oncogenic (high risk [hr]) human papillomavirus (HPV) subtypes, is the most common cancer in women in Guatemala and the most common cause of cancer mortality in women aged 15-44 years. Visual inspection with acetic acid (VIA) with onsite cryotherapy "test-and-treat" is recommended for underserved Guatemalan indigenous rural women. This research assessed: 1) hrHPV infection prevalence in women screened by VIA; 2) Sensitivity and specificity of VIA in detecting hrHPV infection and cytologically identified precancerous and cancerous lesions; and 3) Factors associated with hrHPV infection. Analysis of anonymous data collected during VIA clinics in 2013 ($N = 205$) and 2017 ($N = 234$) for indigenous women aged 21-65 years in six villages showed 22.6% (95% confidence interval [CI]=18.7%-27.2%) had hrHPV cervical infection. VIA results were abnormal in 5.9% (95%CI=3.8%-8.8%). Only nine VIA exams in 89 women with hrHPV were abnormal (Sensitivity=10.1%, 95%CI=4.7%-18.3%), although abnormal VIA was associated with hrHPV (Prevalence

Ratio [PR])=1.8; 95%CI=1.1-3.1; *P*=.05). Of 221 women who had VIA, hrHPV nucleic acid testing and liquid preparation cytology (equivalent to Papanicolaou or "Pap") testing, 10 (4.7% [95%CI=2.3%-8.5%]) had abnormal cytological results, including one cancer, four high- and five low-grade squamous intraepithelial lesions. VIA sensitivity and specificity for detection of precancerous cytological abnormalities and cancerous lesions were 20.0% (95%CI=2.5%-55.6%) and 96.0% (95%CI=92.3%-98.3%) respectively. In contrast, hrHPV sensitivity and specificity were 100% (95%CI=71.7%-100%) and 88.7% (95%CI: 83.9%-92.7%). In both years combined, women aged fewer than 29 years or reporting fewer than four pregnancies were more likely to have hrHPV cervical infection (36.8%, 27.3%, respectively) than those who were older or reported more pregnancies (18.7; <.0001 and 17.9%; *P*=.025, respectively); 60.0% reported some form of modern contraception. Progesterone injections or implant users were more likely to have hrHPV infection (31.9%) than women using other or no contraceptives (19.5%); PR=1.6; 95%CI=1.1-2.4; *P*=.01). These data suggest that VIA may not be sufficiently sensitive for use in cervical cancer screening. "Test-and-treat" screening using hrHPV real-time testing, as recommended by the World Health Organization may be preferable to VIA, and may be acceptable using self-collected specimens.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

ABBREVIATIONS

ASC-H	Atypical squamous cells, cannot exclude high grade lesion
ASC-US	Atypical squamous cells, undetermined significance
CIS	Carcinoma in-situ
HPV	Human papillomavirus
hrHPV	High-risk human papillomavirus
HSIL	High-grade intraepitheilial lesion
INCAN	Instituto de Cancerologia, Guatemala's National cancer hospital
LAC	Latin America and Caribbean region
LEEP	Laser loop electrosurgical excision procedure
LMIC	Low- and middle-income countries
LSIL	Low-grade intraepitheilial lesion
MOH	Ministry of Health
NGO	Non-governmental organization
NILN	Negative for intraepitheilial lesion
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
STI	Sexually transmitted infection
VIA	Visual inspection with acetic acid
VILI	Visual inspection with Lugol's iodine

I. INTRODUCTION

Background

Cervical cancer is one of the most studied and best-understood malignancies. Cancer producing ("high-risk" or oncogenic) human papillomavirus (hr-HPV) subtypes are recovered in 95%-100% of cervical cancer specimens and hrHPV infection has been identified as a necessary element in most cases of cervical carcinoma[1-11]. Although cervical cancer is one of the most preventable and treatable cancers, it remains a leading cause of cancer mortality in women worldwide[2,4,6-9,12-16]. In high-income countries, cervical cancer morbidity and mortality have dramatically declined due to the use of cytological screening with Papanicolaou (pap) "smears" and timely treatment. Cervical cancer screening with cytology has been considered standard-of-care for decades; at least 75% of women participate in cervical cancer screening in high-income countries[15]. At present, 81%-87% of new cervical cancer cases and deaths are believed to occur in low- and middle-income countries (LMICs)[2,4-9,12-16], in which women have limited access to needed screening. Guatemalan indigenous women are at particular risk for cervical cancer due to their marginalization, yet there is no registry for indigenous people and cancer rates in Guatemalan indigenous people, who represent greater than 50% of the population[17].

Statement of the Problem

The Latin American and Caribbean (LAC) region is home to just nine percent of the world population, but bears approximately 16% of the world burden of cervical cancer mortality[18,19]. Lack of infrastructure to provide care, including screening and follow-up, and poverty, creating marginalized populations in low-income countries,

perpetuate barriers to health care access. According to a 2011 Pan-American Health Organization (PAHO) report "by the year 2030, cervical cancer is expected to kill over 474,000 per year, 95% of these deaths in low-and middle income countries[20]", an apparent result of low access to screening, diagnosis and effective treatment. Guatemala is a middle-income country plagued by inadequate healthcare infrastructure, endeavoring to provide service to remote indigenous populations. In LMICs, a highly promoted strategy for low-cost cervical cancer screening is visualization and inspection with acetic acid (VIA). VIA is often performed as part of cervical examination with magnification using colposcopy. In LMICs, it is used in conjunction with immediate treatment with cryotherapy in a "see-and-treat" exam. Colposcopy uses magnification for biopsy and curettage of tissue within the cervical os. The biopsies and endocervical tissue from the curettage are examined for cellular changes. The results of the tissue histopathology guide treatment decisions. In contrast, VIA relies exclusively on examiner's naked-eye assessment, so training is vital. The judgment to treat is based on the unmagnified exam, with no laboratory validation. Cryotherapy as a treatment for precancerous lesions is not entirely without risk, but generally is well tolerated. Initial side effects can include watery discharge, pelvic pain, vaginal bleeding and potentially infection. Risks include infection, obliteration of the cervical transformation zone and stenosis of the cervical os, complicating future evaluations. The benefit of immediate treatment may be outweighed by the difficulty in establishing continuity of care, and follow-up over years, an essential component that has decreased mortality in high resource countries.

Significance of the Study

Little is known of the prevalence of high-risk HPV subtype infection in rural indigenous women in Guatemala[21]. or the effectiveness of low-cost screening techniques, such as VIA, currently used to identify women with precancerous and cancerous lesions[22-24]. However, there is growing concern about the *acceptability* and *efficacy of VIA in Guatemala***,** due to country-specific training and competence issues and issues related to VIA itself[24].

Current Study Aims

The objectives of this research are to:

1. Estimate the prevalence of high-risk anogenital HPV-subtype cervical infection in Alta Vera Paz, rural Guatemala;
2. Evaluate the sensitivity and specificity of VIA in detection of clinically significant cervical infection with high-risk anogenital HPV-subtypes using as the gold standard nucleic acid high-risk subtype-specific HPV testing and liquid-based cytology (equivalent to Papanicolaou or "Pap smears");
3. Identify factors associated with hrHPV infection in the indigenous female population in the Alta and Baja Vera Paz regions of Guatemala.

The overarching goal of this research is to evaluate the gaps between screening and effective treatment of precancerous and cancerous lesions in rural Guatemala, interventions to eliminate those gaps and efforts to reduce the substantial preventable HPV-associated mortality in the region. Evaluation of VIA requires simultaneous collection of samples HPV DNA hybrid testing, cytology and or biopsy, and the

laboratory technology to conduct these tests. Lack of laboratory availability is already identified in Guatemala as a deficit, and the healthcare infrastructure has no method for follow-up[24] or patient tracking. Therefore there has been little opportunity to validate the results of VIA in rural Guatemala and determine if the exam is likely to reduce the mortality associated with cervical cancer.

Hypotheses: Based on literature review and preliminary studies, we hypothesized that:

1. High-risk HPV-subtype cervical infection prevalence in the study population will exceed 20%.
2. "Real world" sensitivity of VIA to detect active infection with high-risk HPV subtypes will be lower than published estimates of 30%-40%.
3. Cervical cancer screening in rural Guatemala with HPV testing would be improved by hrHPV testing with onsite same-visit ("real-time") results.

This research compared VIA results to hrHPV and state-of-the-art cytological testing and assessed the prevalence of hrHPV infection in the Vera Paz regions in Guatemala. Analysis of de-identified data collected during VIA clinics during which hrHPV testing was performed allowed assessment of hrHPV prevalence, and estimation of sensitivity and specificity of VIA screening vs. hrHPV testing and cytological evaluation. This study assessed prevalence in the Vera Paz region of Guatemala. Previous to this research, a study by Valles[25] et al in 2009 in Escuintla, Guatemala, showed high hrHPV prevalence.

A secondary analysis of data collected to compare VIA with cytology and hrHPV screening assessed the sensitivity and specificity of VIA during free VIA clinics in rural communities in the Vera Paz regions of Guatemala. There have been no previous studies

of this kind assessing VIA in the field in rural Guatemala indigenous persons outside of urban settings.

The referral of patients to specialized diagnostic and treatment facilities is informal, and is reliant on patient initiative and adherence. Chary[26], 2015 published her study following women within INCAN (Instituto Nacional de Cancerología [National Institute of Cancer] the National Cancer Hospital in Guatemala City), as they navigated their way through the system for care. Rural indigenous women have identifiable barriers to general and particularly specialized care including travel time and cost, loss of work and care of family during absence; this unique community has cultural perspectives and historical relationships with the non-indigenous population that must be considered. Even once in care, equipment and medications are often unavailable, broken or too expensive.

Memory of Silence, a document mandated by the Peace Accords in 1994, was completed by the Commission for Historical Clarification, an impartial record, obtained through survivor testimony, of the human rights abuses perpetrated upon the Mayan people and communities over the three decades of civil conflict in Guatemala[27]. The document describes the military dictatorship's agenda of instilling fear of the government and exerting control of Mayan communities through use of torture, rape and disappearances, sometimes forcing indigenous persons to perform these acts against other community members. Loss of the communities and culture is evident today in the decreased use of traditional dress, an external declaration of ethnicity. The Justice system lost credibility as it ignored human rights violations inflicted on civilians by the Guatemalan military.

The deep shortage of resources and opportunity in Guatemalan rural indigenous communities reflects worldwide historical and contemporary inequities[28]. Discrimination toward the indigenous people and poor access to preventive health care are compounded by the gender inequality and violence experienced by indigenous women[29] which are associated with increased risk for cervical cancer[30].

References

1. Koutsky L, Galloway D, Holmes K. Epidemiology of genital human papillomavirus infection. *Epi Review* 1988; 10:122-163

2. Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. *Gyn Onc*. 2008; 110 s4-s7

3. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J of Clinical Virology* 32S. 2005; S16-S24

4. Schiffman M, Doorbar J, Wentzensen N, de Sanjose S, et al. Carcinogenic human papillomavirus infection. *Nature Review/Disease Primer.* 2016; 2: 1-20

5. Dailard C. The public health promise and potential pitfalls of the world's first cervical cancer vaccine. *Guttmacher Policy Review* 2006; 9/1.

6. Thun M, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. *Carcinogenesis*. 2010; 31:100-110.

7. Bray F, Jermol A, Grey N, Forman D. Global transitions according to Human Development Index (2008-2030): a population-based study. *Lancet*. 2012; 13: 790-801

8. De Sanjose, S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* 2010; 11: 1048-1056.

9. Campos, NG, Tsu V, Jeronimo J, Mvundura M, Lee K, Kim JJ. When and how often to screen for cervical cancer in three low- and middle-income countries: A cost-effectiveness analysis. *Papillomavirus Research* 2015; 1:38-58.

10. Burchell AN, Winer RL, de Sanjose S, Franco EL. *Epidemiology and transmission dynamics of genital HPV infection. Vaccine.* 2006; S3/52-S3/61

11. Burd E. *Human papillomavirus and cervical cancer*. Clin Microbiol Rev 2003; 16:1-17.

12. Ansink AC, Cervical cancer in developing countries: how can we reduce the burden? Awareness raising, screening, treatment and palliation. *Trop Doc.* 2007; 37: 67-70.

13. Yang B, Bray F, Parkin DM, Sellors JW, Zhang Z-F Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost Int. *J. Cancer*. 2004; 109:418-424.

14. Chamot E, Kristensen S, Stringer JSA, Mwanahamuntu M. Are treatments for cervical precancerous lesions in less-developed countries safe enough to promote scaling-up of cervical cancer screening programs? A systematic review; *BMC Women's Health* 2010; 10:11

15. Tsu ND, Levin CE. Making the case for cervical cancer prevention: what about equity? *Reprod Health Matters.* 2008; 16:104-12.

16. Denny L Cervical cancer prevention: new opportunities primary and secondary prevention in the 21st century. *Int J Gynaecol Obstet*. 2012;119: S80-4.

17. Moore, S, Forman D, Pineros M, Fernandez SM, de Oliveira Santos M, Bray F. *Cancer in indigenous people in Latin America and the Caribbean: a review*. Cancer Medicine. 2014; 3:70-80

18. Arrossi S, Sankaranarayanan, R, Maxwell D. Incidence and mortality of cervical cancer in Latin America. *Salud Publica Mex*. 2003; 45:5306-5314.

19. Bruni L, Diaz M, Castellasque X, Ferrer E, de Sanjose S. Cervical Human Papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis.* 2010; 202: 1789-99

20. Cervical Cancer Action, Progress in Cervical Cancer Prevention: the CCA Report Card 201. http://www.paho.org/hg/divdocuments/2011/CCA_reportcard_low_respdf#page=3Jzoom=page_actual,-4-79 accessed May 6, 2017

21. Bruni L, Barrionuevo-Rosas L, Albero G, Mena M, Gomez D, Munoz J, Bosch FX, de Sanjose S. *ICO Information Centre on HPV and Cancer (HPV Information Center). Human Papillomavirus and Related Diseases in Guatemala*. Summary Report 19 April 2017

22. Lazcano-Ponce E, Alonso P, Ruiz-Moreno JA, Hernandez-Avila M. Recommendations for cervical cancer screening programs in developing

countries. The need for equity and technological development. *Salud Publica de Mexico.* 2003; 45: supl 3

23. Agurto I, Sandoval J, de la Rosa M, Guardado, E. Improving cervical cancer prevention in a developing country. *Inter J for Qual in Health Care* 2006; 18: 81-86

24. Chary AN, Rohloff P, Major challenges to scale up visual inspection-based cervical cancer prevention programs: the experience in Guatemala; *Glob Health Sci Pract.* 2014; 2: 307-17

25. Valles X, Murga GB, Hernandez G, Sabido M, Chuy A, Lloveras B, Alameda F, de San Jose S, Bosch X, Perdoza I, Castellsague X, Casabona J. High prevalence of human papillomavirus infection in the female population of Guatemala *Int J Cancer* 2009*;* 125:1161-1167.

26. Chary A. Structural inequities in the hospital system: rural and indigenous women's experiences of cervical cancer treatment in Guatemala. *Revue Sociologic Sante*; 2015; 38: 161-177

27. Guatemala: Memory of Silence. Report of the Historical Clarification Commission Washington DC: American Association for the Advancement of Science 1999 Retrieved from http://hrdag.org/wp-content/uploads/2013/01/CEHreport-englis.pdf

28. Gaikidou E, Nordhagen S, Obermeyer Z. Coverage of cervical cancer screening in 57 countries: low average levels and large inequities. *PLoS Med.* 2008; 5: e132

29. Ogrodnik C, Borzutzky S. Women under attack: violence and poverty in Guatemala. *J of Inter Women's Studies*. 2011; 12:55-67

30. Coker AL, Hopenhayn C, DeSimone CP, Bush HM, Crofford L Violence against women raises risk of cervical cancer *J Womens Health*. 2009; 18:1179-85.

II: LITERATURE REVIEW

Theoretical Foundation: Theory of Gender and Power

The theories that inform this analysis are the Theory of Gender and Power[1] (Figure 1) and the Theory of Planned Behavior (Figure 2)[1]. Women in Guatemala have

Figure 1: The Theory of Gender and Power describes the unequal milieu that characterizes rural indigenous women in Guatemala, in which women incur higher risk of cervical cancer, and try to seek health care while coping with high illiteracy rates, poverty and poor resource access.

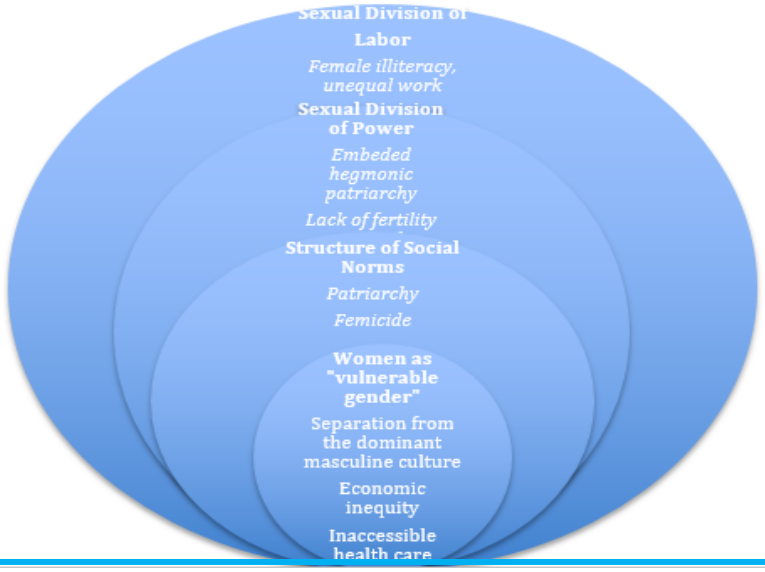
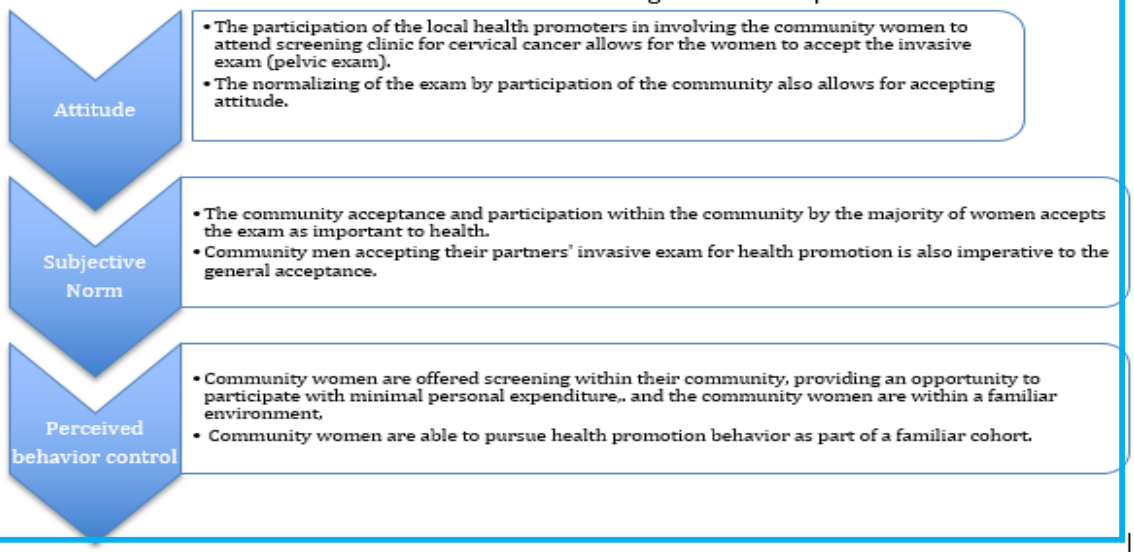
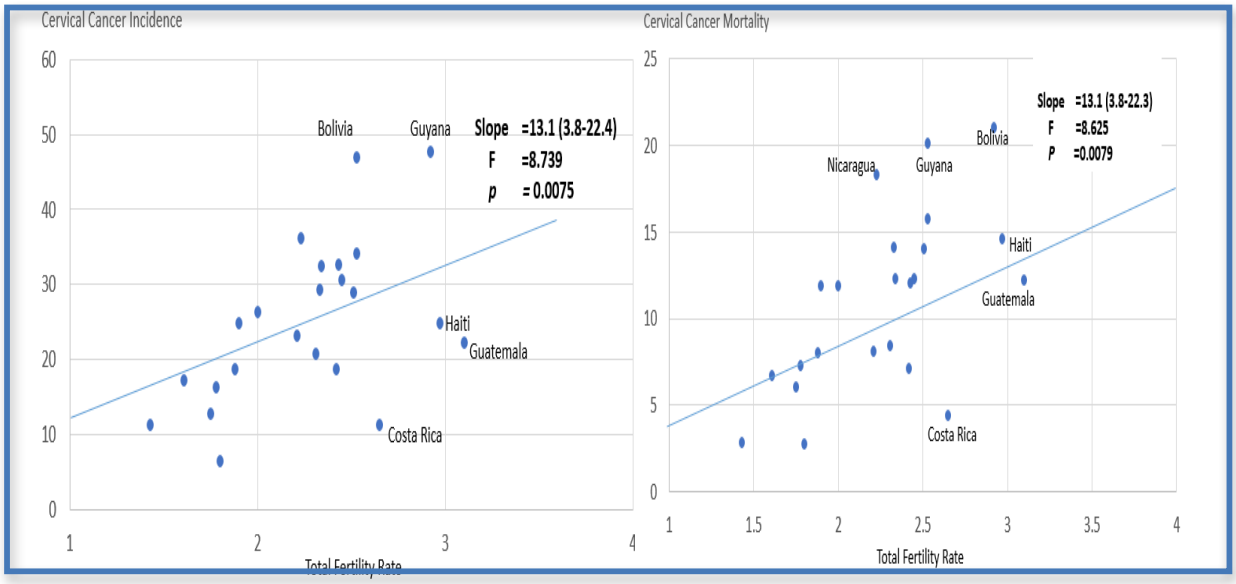


Figure 2 Theory of Planned Behavior: With perceived control over the opportunities, resources and skills needed to affect behavior, intentions can evolve when rural communities have easily accessible health care. Attitudes related to healthcare-seeking behaviors include expectations of outcome and value of the expected outcomes. The subjective norms and perceived beliefs of others promote the desire to comply with the community norms of healthcare-seeking behavior and become intention to obtain cervical cancer screening and follow-up.



long been subject to male dominance, and to a lack of women's health infrastructure and contraception services[2]. The Theory of Gender and Power examines a woman's capacity on three levels. The first (Division of Labor), in Guatemala strongly favors men. Income inequality is related to education inequity[2,3] only 39.9% of rural indigenous women are literate and of those, nearly 80% have attended school an average of three years[3,4] while 53.3% of rural indigenous men are literate[3]. Guatemalan women bear most of the responsibility in raising children and Guatemala has one of the highest fertility rates in Latin America and the Caribbean (LAC) (Figure 3)[5]. Total fertility rate is directly and

Figure 3. Cervical cancer incidence and mortality, by total fertility rate, 23 selected countries, Americas Global Initiative for Cancer Registry Development, 2012, and Total Fertility Rate, WHO.



strongly significantly associated with cervical cancer incidence (p = .0075) and cervical cancer mortality (p=.0079).

Childbearing and childcare responsibilities also limit their access to employment and higher parity is associated with increased risk for cervical cancer[6,7]. Poverty is a

recognized risk factor for cervical cancer[8,9], and nearly 71% of the rural population of Guatemala lives in poverty8.A majority of indigenous women work in home farming, a primary source of food and income, yet only 26% farm family-owned land – and that is generally owned with their husband[10]. Farming and care of animals and wood gathering are primarily women's responsibility, not income generating. It is estimated that women contribute four-fold what men contribute in domestic work[3], 86% of households rely on wood as fuel, which contributes to the degradation of natural resources, perpetuating the poverty cycle. Wood burning for cooking exposes the women to smoke, which is suspected of not only promoting respiratory illness but also increasing risk of cancers[10].

The Sexual Division of Power in the theory is manifested by the violence against women in Guatemala. The death rate resulting from violence against women in Guatemala, the third highest in Latin America in 2012[11-13], perpetuates a vulnerable female society. The Guatemala Human Rights Commission has investigated the roots of this violence, with the civil war contributing a component[11]. Indigenous women suffered extreme violence at the hands of the government forces[12-14]. During the civil war, rape and torture were used to terrorize the indigenous population[13-16]. Contemporary research indicates organized crime and gang activity also perpetrate violence against women[2], perpetuating misogynistic attitudes. These are exacerbated by government indifference in prosecuting these crimes[2,11,12] continuing the cycle. It is estimated that partner violence[2,11,12] is an important cause of femicide (gender-based homicide). Domestic violence was the suspected cause of 61% of the femicides in Guatemala in 2008[11]. Perpetrators of violence against women have enjoyed impunity, with less than 2% of cases involving violent deaths of women prosecuted between 2005 and 2007[2,11,12]. Child

abduction and forced prostitution of girls and women also contributes to the violence against women and increased risk of cervical cancer[2,16,17].

The Theory of Gender and Power offers a core interpretation of relationships within a culture based on the sociopolitical concept of gender, as distorted by combined forces of government-endorsed, extrajudicial and criminal violence[1,17,18] (Figure 1). This structure controls opportunities afforded to women, and their subjugation within communities that are themselves are intensely subjugated. Community structures establish social norms and religious beliefs that serve to control relationships within patriarchal frameworks[1]. The restrictions of working within the home to support the family, and selling produce in the local markets limit women's economic power. Lack of health care access and negative societal concepts regarding contraception (religious prohibitions, and understanding of contraception as a form of government population control in the context of genocidal efforts) may be constraining forces on women's personal fertility control and autonomy. Infrastructure deficits in health care access constitute an institutional discrimination, accepting indifference to women. Unprosecuted femicide tacitly communicates the appropriateness of violence towards women, and misogynistic paradigms isolate women from justice and political power. Indigenous people have struggled to gain respect and trust the government following the civil war, but there is deep-seated mistrust of governmental services[14,15], both judicial and service-related, that isolates indigenous women from self-realization. Women in Guatemala have multiple challenges in seeking health care: poverty, isolation, disrespect of their ethno-identification and deficient social programs to promote literacy and economic security[8].

Theory of Planned Behavior

The Theory of Planned Behavior (Figure 2) explains issues related to women's ability to learn about and incorporate cervical cancer screening into their lives, and how the community of women at risk accepts pelvic exam, and understands and acknowledges personal risk. The Theory of Planned Behavior[1,19,20] is an extension of the Theory of Reasoned Action[21] and posits that intentions predict behaviors, that is, intentions are predicted by attitudes toward the behavior and subjective norms about the performance of the behavior, and extends the Theory of Reasoned Action to include perceived behavioral control. Specifically, the Theory of Planned Behavior suggests that intentions are predicted by attitudes toward the behavior and subjective norms about the performance of the behavior[22]. The Theory of Planned Behavior suggests that, provided support for their health care seeking, and educational efforts to encourage self-protection, if not self-realization, women can change the subjective community norm.

Low levels of control may politically and economically increase indigenous Guatemalan women's risk not only for physical violence but also for conditions like reduced access to cervical cancer screening, diagnosis and effective treatment[23]. Understanding the communities, power structure, and health behaviors related to access and education can open the dialogue to health literacy, but the overwhelming poverty must be addressed to provide greater autonomy to the people and women in particular. Paul Farmer writes *"…inequities have powerfully sculpted not only the distribution of infectious diseases, but also the course of disease in those affected"*[23]. Writing about emerging infectious diseases, his statements apply to cervical cancer, recently recognized

to be a chronic infection, "*An implication, clearly, is that one place for disease to hide is among poor people, especially when the poor are socially and medically segregated*"[23]. This certainly typifies the situation in Guatemala, where death from cervical cancer is not recorded in vital records[24,25] and the actual toll on women is extrapolated, not determined. Reliance on health promoters from within indigenous communities provides a construct through which women can begin to understand the concept of screening for cervical cancer, and normalizes pelvic exams[26]. As rural indigenous women are educated to understand the risk of cervical cancer, they can accept and promote screening, as access is made available through the NGOs offering care. This, in the lens of the Theory of Planned Behavior, evolves into intention to seek care through improved access, and community acceptance of the exam, facilitating for the health care seeking behavior.

Cervical cancer is a daunting burden on both social and economic sectors of Guatemala. Control of cervical cancer in Latin America has been for the most part unsuccessful[9,27]. Herrero et al.[28] estimate that even if incidence rates are not increased, there would be an increase in case numbers solely based on the population aging. The screening programs that have curtailed cervical cancer deaths in high-income countries involve technical procedures, laboratory access, and consistent follow-up generally regarded as too complex and expensive for poor communities countries like Guatemala indigenous populations[8]. Nevertheless, the success of antiretroviral scale-up in low- and middle-income countries worldwide, with plunging HIV-related mortality rates, particularly in LAC, have sparked interest in offering technically complex interventions in these countries, particularly to cancer patients[29-32]. Pursuit of technically advanced but highly acceptable women's health care and cervical cancer prevention and treatment in

Guatemala should be pursued, within the context of the experience of the indigenous women. Attentiveness to their need to have an understanding of the process respectfully provided in their language, and with consideration of the historical and cultural gender discrimination suffered, suggests that help must be sought from within the communities to introduce the most effective interventions in the most acceptable way.

The Theory of Planned Behavior builds a base of education and local support for women to structure an understandable and practical design for provision of their care. As noted in rural Peru[33] predictors for participation in screening programs included: knowing a woman who had participated in VIA, having a supportive partner, having attended an educational session concerning cervical cancer and having a satisfactory experience with the health care system. Deterrents to participation included having a partner who does not condone the exam, a poor experience interacting with the health care system, and belief the exam was detrimental to her.

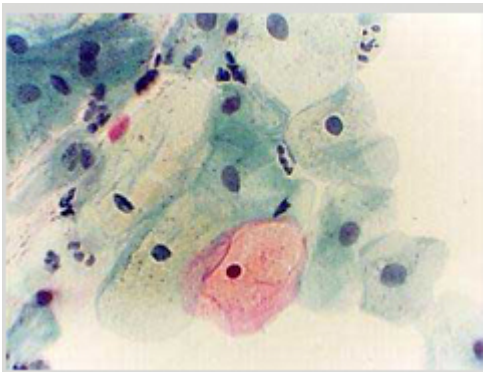
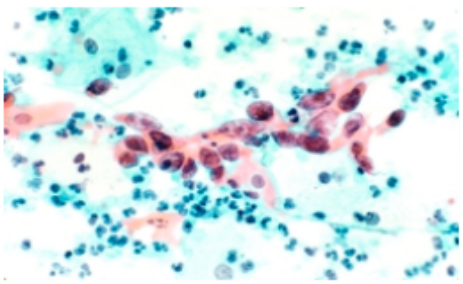
The lack of control politically and economically[2, 34] increases indigenous Guatemalan women's risk for not only for physical violence but also for reduced access to cervical cancer screening, diagnosis and effective treatment. Understanding the communities, the power structure, and health behaviors related to access and education can open the dialogue to health literacy, but the overwhelming poverty and unacceptably low access to care must be addressed[18,36-37].

Natural History of Human Papillomavirus (HPV) Infection

There is an abundance of literature describing the natural history of HPV since even before the association/presence of HPV was noted in cervical cancers, when screening techniques such as the Papanicolaou cytology ("pap smear") and diagnostic

approaches (e.g., biopsies under colposcopy) first suggested the sexually transmitted nature of cervical cancer[38,39]. The current understanding of the natural history of HPV further informs the protocols used in screening. The Bethesda terminology[38] classifies the lowest level of high-risk hrHPV subtype infection detectable by cytology (microscopic assessment of samples obtained by pap or liquid preparation) as the squamous intraepithelial lesion (SIL), low or high-grade, and describes the progression of lesions to carcinoma in situ, defined as malignancy before metastasis (malignant spread) **(**Table 1**).**

Table 1 *2014 Bethesda System for Reporting Results of Cervical Cytological Examination*

Squamous Cell: NILN: Negative for intraepithelial lesion ASC-US - Atypical squamous cell undetermined significance, reported with hrHPV +/- ASC-H - Atypical squamous cells, cannot exclude high grade lesion LSIL – Low grade intraepithelial lesion, mild dysplasia/CIN 1 HSIL – High grade intraepithelial lesion. Moderate/severe dysplasia/ CIN 2, 3 CIS – Carcinoma in situ SCC – Squamous cell carcinoma Glandular Cell: Atypical - Endocervical cells – favor neoplastic, glandular cells – favor neoplastic Endocervical adenocarcinoma in situ Adenocarcinoma- specified as endocervical, endometrial, extrauterine.	NILN  SCC  Cytological specimen showing cervical cancer, specifically squamous cell carcinoma in the cervix. Tissue is stained with pap stain and magnified x200. Source: NCI Visuals Online.

of squamous cell or endocervical adenocarcinoma type[38]. An initial cellular change, atypia, is a diagnosis for which pathological determination of cause by HPV co-testing is recommended and if positive, closer follow-up is appropriate. This grading of lesions requires laboratory sophistication and technique rarely available to women in rural areas in low- and middle-income countries (LMICs); in high-income countries, the grading and location of the lesion dictates the treatment protocol[39]. LSIL is considered an acute infection phase, often cleared by immunocompetent women, while HSIL represents a chronic infection and cellular changes, precursor to cancer. Gavitt and Winer[40] examined serum antibodies along with vaginal/cervical HPV detection; their research supports a reactivation of prior infection in older women rather than newly acquired infection. They also found an association of viral detection with the menstrual cycle, suggesting potential effects of menopause on reactivation of the virus[41].

Over 100 subtypes of HPV exist, 40 of which are associated with the anogenital tract[41-49]. "High risk" (hrHPV) subtypes, those that can cause malignancies, can establish persistent infections, causing a disruption of the normal maturation of cells in the epithelial layers by proliferation within the squamocolumnar junction (SCJ)[40-44,50] (Figure 4). The SCJ, is also called the transformation zone, where epithelial cell transition occurs, from resistant squamous cell epithelium (consisting of multiple layers of tough cells) to the much more susceptible single-cell layer of columnar cells. Over 90% of lower genital tract cancers arise in the SCJ (Figure 4)[39,50]. It is thought that the virus enters basal cells below the columnar cells via micro-abrasions. As HPV infects the cervical cells, it causes the host to replicate HPV. With the help of the host cells' normal shedding, more viral particles are released[39-42]. Once integrated into the host, these genes

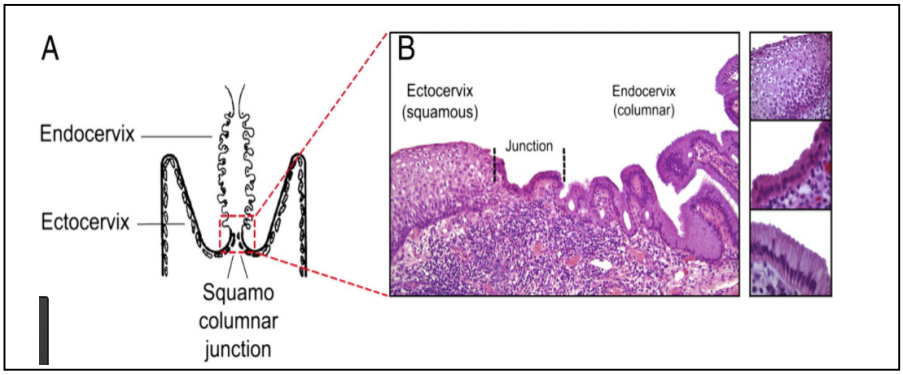


Figure 4. Coronal section diagram of cervix (A) and microscopy (B) from Herfs et al. *PNAS.* 2012; 10516-21.

enter basal cells below the columnar cells via micro-abrasions. As HPV infects the cervical cells, it causes the host to replicate HPV. With the help of the host cells' normal shedding, more viral particles are release[39-42]. Once integrated into the host, these genes disrupt normal cells by binding to and inactivating tumor suppressor proteins[39-42]. Fifteen HPV subtypes are classified as oncogenic; HPV 16 is thought to be present in nearly 50% of cervical cancers and HPV 18 is responsible for an additional 10%-15%[39-42,48,49]. Most women with normal cell-mediated immunity clear the active infection but the infection can persist latently for decades[42-46]. The typically years-long natural history of oncogenic HPV subtype infection in immunocompetent persons, and its typically slow progression to malignancy, allows for intermittent screening. Generally, women aged mid-thirties to fifties begin to exhibit cellular changes that progress to cancer. The general population testing in Escuintla department in Guatemala by Valles[25] in 2009 demonstrated an increase in hrHPV infection in women over 35 years of age. Such changes are thought to be related to reactivation of the virus associated with changes

related to menopause or immunity and even potential exposures by either new sexual partners or long-term partners' new sexual partners[40]. Cervical cancer is associated with persistent infection, the course of which is influenced by viral genetics, host immune response and related cofactors (e.g., HIV infection, malnutrition)[42]. Behavioral factors are known to have the least influence with only a 1.5-2 fold increase in developing pre-cancer after an infection with hrHPV Once integrated into the host, these genes disrupt normal cells by binding to and inactivating tumor suppressor proteins[39-42]. Once integrated into the host, these genes disrupt normal cells by binding to and inactivating tumor suppressor proteins[39-42]. [52-58]. Considering the modest impact of behavioral risk factors, a woman's risk of progression to cervical cancer is dependent upon not only her behavior but that of her husband or partner's.

The rapid acquisition of infection with an anogenital HPV subtype following sexual debut[59-62], 28.5% of women test positive for hrHPV subtypes within one year of their sexual debut[61], suggests that screening and treatment programs must address the latency and potential reactivation of the infection. The reality is that most HPV infections are spontaneously resolved[39,43-45,63-65] and do not reactivate and progress to cervical cancer, suggesting that overtreatment may occur if cytology and determination of the degree of lesion to be treated are unavailable. The mere presence of hrHPV in the genital tract does not in itself suggest inevitable progression to cancer for young immunocompetent persons, which again points to the importance of infrastructure that allows for follow-up, and laboratory sophistication to "grade" a lesion prior to treatment[35,64,65].

Evaluation of Cervical Cancer Screening Tests

Screening is a process designed to detect disease processes before they become symptomatic, and typically precedes a definitive diagnosis[66]. Screening tests reduce the number of persons that must be tested to make a definitive diagnosis, generally by identifying a population at higher or highest risk. Screening generally uses a highly sensitive test to identify the sub-population most likely to have the disease process. A more specific test to identify the "true" cases of the condition ("confirmatory" test) often follows the screening tests. Screening tests are evaluated by what percent of persons with a positive "gold standard" test they detect (test "sensitivity") and "specificity" (the percent of persons in whom a "gold standard" test has ruled out the condition that test negative on the screening test). Sensitivity and specificity are test characteristics. The predictive value of a positive or negative screening test is affected by the prevalence of true positives in a population. The predictive value of a negative test (proportion of negative screening tests that occur in persons that are truly negative) and predictive value of a positive test (proportion of positive screening tests that occur in persons that are truly positive) assess screening test performance at specific population levels. In populations with very low prevalence of the condition for which persons are being screened, even tests with very high specificity will have low positive predictive values.

Cervical Cancer Screening in Low Income and Middle Income Countries

To assess and address cervical cancer in underserved and low-resource populations, VIA has been proposed[28,64,65, 67-71]. Hinselmann, who experimented with VIA in 1924, first studied the cervix using acetic acid, which is known to whiten

precancerous and cancerous lesions[72]. VIA developed into colposcopy when magnification improved the technique. During colposcopy, biopsies of lesions are taken for diagnosis and to "stage" lesions, determining disease progression, as Hinselmann's research demonstrated that visualization alone was not sufficient to diagnose precancerous lesions or cervical cancer[71]. This technique is used to confirm pathology and direct treatment as needed. Colposcopy became widespread in medical practice in the 1960s-70s. In the 1990s found many LMICs resorted to VIA as a screening technique, revisiting historic development of screening approaches of cervical cancer. The minimal need for equipment and immediate results were believed to meet the needs for screening for low-resource settings. However the accuracy of VIA remains difficult to assess.

Denny et al.[73] in 2002 compared VIA to VIA with magnification and cytology in South Africa. There was minimal difference in sensitivity of direct visualization and enhanced view with magnification. Both had negative predictive values of 94.4%-97.2% in all lesion categories (high- or low-squamous intraepithelial lesion) as did cytology (94.5%-96.5%). The positive predictive value of the cytological examination was 34.5%, whereas VIA had a positive predictive value of 12.9%-13.7%. The subjectivity of the test, improper application of acetic acid and poor visualization of the cervix were offered as explanations for lesion detection failure in VIA. In 2005, Sarian, et al.[74] as part of the Latin American Screening study assessed the performance of VIA and against colposcopy found a sensitivity of 45.4%-50% for HSIL. The study included hrHPV testing, and in conclusion discussed augmenting VIA with hrHPV screening, following up women testing positive with colposcopy. The authors advise that "there is no argument that organized cytology screening is the only cost-effective means of cervical

cancer control" pointing to the need to explore ways to improve access to cytology screening within a context linkage of patients with suggestive results to specialized care. In Peru, Jeronimo et al.[69] in 2005 compared VIA to pap smear using colposcopy as the gold standard, and determined positive predictive value of both VIA and pap smears to be under 10%. It is noted that the providers performing the pap and VIA exams were oncologists[69]. Yet in the discussion portion, the authors state "VIA…requires a low level of training"[69]. The study does confirm the benefit of immediate results with VIA, noting that 26.3% of women with abnormal pap were lost to follow-up in contrast to 2.3% of women with an abnormal VIA. Herrero et al.[28] examined screening methods in two Latin American countries. In Costa Rica, liquid-based pap, conventional pap and HPV testing were compared; liquid-based pap was shown to have sensitivity/specificity of 87.5%/87.8% compared to pap smear as the gold standard, and 63%/93.7% and HPV testing at 85.3%/88.2%. All the tests had a >99% negative predictive value, with positive predictive value of 11.5% for conventional pap, 8.5% for HPV testing and 9.0% for the liquid-based pap. Costa Rica developed a National Cytology Laboratory during the reorganization of the cervical cancer-screening program and since noted a decline in incidence and mortality from cervical cancer. Costa Rica committed resources into establishing infrastructure for diagnostics and treatment, which improved continuity of care. In Peru, VIA had similar results: HPV testing was the most sensitive (89.7%) however prevalence of abnormal cytology was noted to increase with age while hrHPV positivity decreased. Examiners were trained midwives, and over the course of the study, false positive VIA decreased with experience, demonstrating subjectivity in the exam, need for practice, and little evidence of VIA being technically "extremely simple". The

study discussed cytology, hrHPV testing and VIA, and concluded that VIA was appropriate where no other screening options were available but that any program of screening must include referral for specialized care. The study in Peru was part of a project to increase coverage of screening (TATI project[75]). An adjunct of the TATI study by Alamonte[76] was situated in a remote area of Peru, and found that the conditions under which the testing occurred were more apt to reflect the usual conditions in which routine screening was taking place. Both VIA and pap smears performed below expectations compared to other studies; routine cytology (pap smear) was ineffective for screening due to inadequate laboratory quality which also was reflected in the liquid-based cytology. Cytology has been proven in high-income countries to be sufficient for screening, but considerable investment in laboratory facilities and technician expertise must be made before this screening option will be useful in LMICs. Costa Rica is an example of a country where such investment has been made with promising results. Sherris[71] in 2009 reviewed studies that compared VIA with cytology and colposcopy, noting that rural Peru found a sensitivity of 26% for conventional cytology in the region, revealing the unacceptable sensitivity of pap smear for screening in this setting. The Latin American Screening[77] study was conducted in Brazil and Argentina, also comparing VIA with cytology in four cities (three in Brazil and one city in Argentina). Researchers found a similar prevalence of hrHPV in the four cities ranging from 15.4% to 18.8%. There were higher rates of insufficient sampling with the conventional pap compared to liquid-based cytology, which would require a second visit to clinic to repeat the test. The authors advise that "there is no argument that organized cytology screening is the only cost effective means of cervical cancer control" pointing again to a failure of infrastructure. A

meta-analysis by Sauvaget et al.[78] in 2010 reviewed 26 VIA studies in which women had confirmatory tests to determine the accuracy of VIA in screening, finding a sensitivity ranging from 41%-92% (pooled sensitivity=80%). Specificity ranged from 49%-94%. Only two of the studies were in Latin America. The studies that involved VIA compared to colposcopy did not always confirm findings by histopathology, avoiding biopsies of women with negative screening tests. In a study in Nigeria[79], pap and VIA had sensitivities of 60%, and specificities of 100% and 94.9%, respectively. In this study, the risk of overtreatment was preferred over the risk of loss to follow-up, again considering infrastructure as a necessary component in a successful screening program. In Colombia[80], mortality from cervical cancer was evaluated based on five variables: women who reported yearly screening, women who did not receive results of cytology, women with abnormal results who followed up for treatment, women who never had cervical cytology and uninsured women. Increased screening coverage did not decrease cervical cancer mortality in uninsured women. After adjusting for insurance coverage, compliance with follow-up visits was associated with a 40% reduction in mortality. Wright and Kuhn[81] reviewed efforts in LMICs, finding that continuity of diagnosis and treatment was the primary requirement in reducing mortality from cervical cancer and avoiding overtreatment. Studies from India and South Africa reviewed by the authors both indicated less disease progression in women screened and treated, if screened with hrHPV. It was noted that in India there was no reduction in cervical cancer incidence or mortality in the VIA and control arms of this prospective study[81]. A comparison of pap to hrHPV testing to co-testing in the United States by Blatt et al.[82] demonstrated that co-testing in high-income countries would improve diagnosis and treatment. In the results of

this study, 19% of women with cervical cancer would have been missed with hrHPV testing alone. Pap (alone) sensitivity was 91.3%, hrHPV sensitivity alone was 94%, and co-testing had a sensitivity of 98.8%. Utilizing cervical biopsy results, 14.4% of women with abnormal pap had a negative hrHPV test, and of those with biopsies with an HSIL result, 35.4% had cervical cancer. The women who were negative for hrHPV but had high-grade lesions or cervical cancer were older, perhaps representing longer exposure, lower tumor viral levels and underscoring the importance of screening older women at risk for cervical cancer. In another meta-analysis of screening tests for cervical cancer in sub-Saharan Africa, Fokom-Domgue et al.[83] compared VIA with visual inspection with Lugol's iodine (VILI), a solution of potassium iodine, an iodine that turns cervical precancerous and cancer lesions yellow) and HPV testing. Pooled sensitivity for VIA was 82.4% and specificity was 87.4%. VILI sensitivity was higher than VIA and specificity was similar. HPV testing demonstrated a pooled sensitivity of 88.3% and specificity of 73.9%. The screeners preferred VILI, as the cervical mucosal changes were more pronounced without needing reapplication of the iodine and there was no time lapse before evaluation as there is with acetic acid. As with VIA, screening menopausal women with VILI can be inaccurate as atrophied tissue does not take up the iodine. In 2015 Dawood and El-Tahmoudy[84] also compared VIA, VILI and cytology, also demonstrating similar sensitivities and specificities between VIA and VILI, with cytology demonstrating lower sensitivity and higher specificity. Lack of access and follow-up was again identified as a major deficit in cervical cancer control in LMICs. The meta-analysis by Mustafa et al.[85] also compared VIA, cytology and HPV testing, as well as overtreatment compared to under-treatment. Consideration of the future consequences of

over or under-treatment and the context of the woman being tested is an important measure, along with actual mortality. Sensitivity and specificity are important but outcomes have been difficult to measure in many LMICs. Zhou[86] retrospectively evaluated hrHPV test for precision in detecting high-grade cervical lesions as it has been considered first line screening. The sensitivities were similar to prior studies at 80.8% (hrHPV) and 81.2% (pap smear). False negative rates for hrHPV and pap were 8.7% and 9.1% respectively, with co-testing bringing the false negative rate to 1.2%. When considering a screening test and the variables of fieldwork, the improvement in false negative test was important. In Zhou's study, 37% of the women with cervical cancer had been hrHPV negative five years before. In 2017, Raifu[87] evaluated VIA compared to VILI and physicians to nurses as examiners. With both tests VIA and VILI, physician sensitivity was higher than the nurses' and sensitivity decreased with older participants, an effect that has been observed in other studies. Positivity with hrHPV reduced specificity yet improved both nurse and physician sensitivity, even though the result of hrHPV test was unknown to the examiners. Looking at participant factors, Raifu concluded that age, parity, and positive hrHPV increased the accuracy of the examiners. This study indicated that the higher-grade lesion decreased the specificity of the hrHPV screening test, and sensitivity decreased in the older woman screened with visual techniques. Age (older, post-menopausal) and high parity have been identified in studies as factors that increase a woman's risk for cervical cancer[7,88,89]. Age risk is explained by the natural history of hrHPV, the infection taking decades to reactivate, then progress to cervical cancer. Screening menopausal women with VIA is less effective due to the cervical tissue's atrophic changes. As noted previously Guatemalan women have high

fertility rates, a known factor to increased the risk for cervical cancer.

The difficulty in reviewing the accuracy of VIA includes the variations of disease threshold, the standards used for comparison and failure to confirm negative VIA. There can be discrepancies in experience and training of the VIA providers, ranging from physicians with experience in colposcopy to a newly trained non-physician.
VIA does not allow for the grading of a lesion, unlike cytology, and cannot detect the presence of hrHPV subtypes. Aceto-whitening of the cervix can be non-specific, with reparative and immature squamous metaplasia also appearing white[81]. There is no protocol for confirming negative VIA exams, the ability to screen for hrHPV subtypes might enhance or even replace the VIA evaluation and provide better guidance for follow-up for women unable to access the standard cytology screening recommendations. Beyond studies that evaluate VIA in research settings, there has been little assessment in actual use, where problems associated with non-standardization of training, turnover of personnel, subjectivity and unavailable mentoring and continuing education potentially affect the quality of the screening. These factors may reduce the ability of VIA in real-world situations to challenge the encouraging research data endorsing VIA alone as a screening tool.

Treatment in Low and Middle Income Countries

In high-income countries, cervical cancer treatment has moved toward laser loop electrosurgical excision procedure (LEEP), a treatment that uses a small electrical wire loop to remove abnormal cells from the cervix, allowing for assessment of suspicious lesions, grading of the extent of invasive changes and the evaluation of borders to verify the complete removal of lesions[90,91]. High-income countries are actively eliminating

overtreatment by not treating low-grade lesions, observing over time, and treating only high-grade lesions. Cryotherapy – freezing the cervix with a probe to ablate the tissue, allowing for growth of tissue without the infection – was formerly an accepted treatment. It is no longer the treatment of choice in high-income countries due to the effects of freezing on cervical tissue and inability to document complete excision of the lesion and grade the extent of invasion. Cryotherapy causes ablation of the lesion, which may precludes subsequent VIA exams due to scarring of the cervical tissue and the migration of the transformation zone, where the SCJ lies and HPV infection proliferates, and narrowing of the cervical os, which create difficulty in accessing the SCJ for adequate sampling in later screening[70,71,90]. There is a theoretical risk of cervical insufficiency and complications of pregnancy caused by disruption of cervical tissue. Maza[90] discusses quality of gas use in cryotherapy, comparing carbon dioxide (CO_2) to nitrous oxide (N_2O). CO_2 is often easier to obtain; however, impurities can cause inconsistent temperatures and equipment malfunctions. Equipment requires sterilization between women treated, and Maza notes that it is unpredictable in number of patients who can be treated (2-20). Cryotherapy is indicated for treatment of precancerous lesions if endocervical curettage is negative, biopsy confirms disease, invasive cancer is ruled out and the complete lesion is visible. With VIA in LMICs, these treatment criteria may not be well established.

Moreover, the ability to immediately treat with cryotherapy provides a false sense of security, understating the need for continued follow-up and assessment. Schmidt et al.[91] followed a small group of women treated with cryotherapy and found there were recurrences within 2 and 5 years, 24% of which were adenocarcinoma, which is more

difficult to diagnose and in which HPV is not always detectable. Appropriate follow-up with pap smear or biopsy must be incorporated into care, as recurrences should be anticipated if no grading of lesions is done prior to cryotherapy.

The complications of cryotherapy are generally mild, but can include pain, cramping, and watery discharge[91-95]. Women need to be prepared for this and for the recommendation for abstinence from sexual intercourse until healed. Thus, treatment with cryotherapy can contribute to both failure to pursue and difficulty in conducting future screening with VIA due to the cervical changes caused by the freezing. The cervical changes necessitate biopsy and a higher technical level of diagnostic capability that these techniques are designed to avoid[91-93]. Several studies in Africa and Asia have provided significant information regarding the use of VIA and "see and treat" programs offering cryotherapy. In 2012, Ziyauddin and Rajyashiri[93] in India compared cryotherapy to LEEP. As previously noted LEEP requires electricity to perform, as well as costly equipment and a high level of training. It had a cure rate of 94.4% and was useful for diagnosis. In contrast, cryotherapy had a cure rate of 88.2% in this prospective study following women for one year. Even in this relatively short-term study, participants were lost to follow-up and four recurrences occurred in the cryotherapy treatment group of 34. Continued surveillance of treated women is imperative to recognize recurrence, and is a challenge that must be addressed with infrastructure. Cryotherapy is not indicated for treatment of endocervical lesions or glandular disease, neither of which can be assessed by VIA, but which can potentially be obscured by cryotherapy[92]. Failure of cryotherapy can be caused by inadequate freezing of the tissue because of inadequate gas pressure for delivery, poor probe application and insufficient time for freezing[94,95]. Assessment of

impact of VIA and cryotherapy on cervical cancer in Guatemala is desperately needed.

Cryotherapy is not a definitive treatment for cervical cancer, but a treatment for precancerous lesions; therefore, the women treated with cryotherapy as part of a "see and treat" intervention need to be informed that continued surveillance is needed[91-95]. Recurrence at any age is possible. The nature of the chronic infection and progression requires follow-up and continued care, which requires an infrastructure not currently available in rural communities in Guatemala.

The global research conducted to identify strategies to decrease mortality from cervical cancer suggests that VIA is an acceptable screening tool, but the addition of hrHPV co-testing offers improvement in both sensitivity and specificity in testing for risk for cervical cancer and pre-cancerous lesions. No screening can be done in a vacuum; infrastructure to follow-up as needed and to keep records regarding testing and treatment is essential. Catarino et al.[95] discuss the emerging technologies, as they have been trialed and implemented in numerous LMICs, acknowledging that hrHPV may be negative in the presence of a high-grade lesions, and recommend triage of women with positive hrHPV tests. Valles[26] in 2009 estimated that in Guatemala, pap smear coverage was less than 10% of the vulnerable population. Winkler[34] published a study on women's participation in cervical cancer screening in northern Peru and identified four predictors of participation. These were: higher relative wealth; knowing women who have been screened; knowledge and use of the health care system, and having a satisfactory experience within the system[34]. This research supports the need for preventive health education, and community endorsement of participation in screening. Accessing care is a persistent barrier for poor Guatemalan women. The VIA mobile "brigades" have

increased awareness of the risk for cervical cancer. Now, true follow-up for diagnosis and treatment needs to be attainable. Self-sampling for HPV hybrid testing was studied in with indigenous women residing in Santiago Atitlan[96]. Self-sampling, which does not appear to reduce sensitivity, was found to be acceptable to the participants and all indicated they would be willing to periodically provide a sample for testing. While this greatly increases the simplicity of specimen collection, it still also requires a healthcare system that can track patients for continuity of care.

Historical and Political Barriers

Guatemala has significant challenges in health care provision within the rural, largely indigenous communities, with infrastructure deficits traceable through a historical and political lens. Guatemala's history since Spanish colonization is steeped in political conflict and marginalization of the indigenous and rural populations, who suffered economically and witnessed the dismantling of their social/community structures during government-sponsored terrorism over the last three decades[12-15]. Prior to Spanish colonization of the region, Guatemala's population encompassed 30 separate culturally and linguistically distinct subpopulations, most of which share the Mayan cultural identification[12,15,97]. Survival of Guatemalan indigenous people was high, as evidenced by the fact that they remain even after prolonged genocidal efforts, the majority of the Guatemalan population (51%)[12-15]. Linguistically distinct Mayan communities existed separately from the occupying structure, suffering loss of land, the primary source of livelihood and sustenance[12-15]. In the late 1890's the country's economy became internationalized through political negotiations with foreign companies, particularly the

American United Fruit Company[13,98]. This company maintained significant influence and power in the political arena, impacting all aspects of government activity in Guatemala[98].

In the post-World War II era to mid-1950, there was a brief period of representative elected government, labor reform and redistribution of lands to the rural poor[13,15], mostly descendants of the Mayan majority population. This era of reform was brought to an end by a US CIA-backed military coup led by Guatemalan Army General Carlos Castillo Armas[2,13-15] because of concerns of possible Communist involvement in the reforms. The military had long been, and again became, synonymous with the government, suppressing any uprising, but also forcing governmental change through military coups[13-15]. During the Armas tenure, 99.6%[18] of the land that had been nationalized and distributed to Mayans was returned to former landowners, including international companies such as the United Fruit Company. The assassination of Armas in 1957 plunged Guatemala into a violent civil war[13-15,18] which devastated the indigenous populations and rural poor and lasted over 30 years. The complete toll will never be known but an estimated 200,000 people, were killed or "disappeared", over 80% of who were indigenous[13-15,34]. During this time, the government actively suppressed the education of the Mayans and their economic stability. This coincided with government maintenance of the status quo of poverty and illegalization of traditional Mayan healers[99] exacerbating the marginalization and weakening of the culture[12-15].

The Guatemalan Civil War, the longest and deadliest in Central America ended in 1996[12-15]. The Comisión Nacional de Reconciliación (National Commission for Reconciliation) took the first steps toward peace in 1989, but over seven years, fighting continued until the accords that provided for the reconciliation between the armed forces

and guerrilla groups[34,35] and the forging of plans for social and infrastructure reparations aimed at benefiting the poor, marginalized, rural, predominantly Mayan populations. Of the over half of the population in Guatemala documented as indigenous[12,15,97], 90% of the population is classified as poor, or extremely poor[12-15,34]. The indigenous communities endure much shorter life expectancy at birth (17 years shorter than non-indigenous groups).[34]

At the time of the signing of the Peace Accords, the social security system covered only 13% of the populations[34,35,97,99] . This lack of infrastructure precluded the government's accomplishment of the health care improvement provisions of the Peace Accords. Limitations of public health care expenditures were due to conditions of international debt restructuring imposed by the World Bank and International Monetary Fund[35]. The Ministry of Health (MOH) assigned the responsibility for rural health care to NGOs, (an estimated 10,000-15,000) working in Guatemala[99-102]. The Programa de Extensión de Cobertura (Program of Extension of Coverage, or PEC) was instituted in 1997 to improve healthcare access to the rural populations. PEC tried to contract with NGOs already working in Guatemala, many of which declined participation to retain autonomy[35] and limit association with the MOH, a government agency that, due to the long civil war, may have suffered from their association with the acts of the government and by the historical mistrust of rural citizens. Even the philanthropic and religious groups that had constituted the majority of NGOs in Guatemala present during the civil unrest, working in response to natural disasters that plagued the country, tended to be unwilling to accept MOH collaborations for similar reasons.

Since PEC had contracts and funds to distribute, and philanthropic and religious

groups were unwilling to accept them, it turned to an entrepreneurial approach to health care, funding newly contracted (including for-profit) NGOs with little or no experience in rural health care delivery[99-102]. The MOH divided health care into curative and preventive services but did not address infrastructure deficiencies and widespread poverty that contributed to the failure of healthcare system initiatives[15,34,99]. In 2014, all contracts between PEC and health care NGOs were cancelled in accordance with legislation prohibiting the outsourcing of health care. This left a gap in care the country had no immediate measures to replace[103].

According to Instituto Catala d'Oncologia HPV Centre statistics published in 2017, cervical cancer was the leading cause of female cancer and of cancers deaths in Guatemalan women aged 15-44, noting, however, that incidence in Guatemala is extrapolated from surrounding countries[24]. As previously noted, Valles[25] published a study done in Escuintla, a first study in anogenital HPV infection prevalence in Guatemala, comparing HPV in sex workers recruited from a sexually transmitted infection (STI) clinic with general population HPV prevalence in women attending a community clinic. He found prevalence rates of 38.1% within the general population, and 67.3% among sex workers. The prevalence of HPV for sex workers declined with age, a trend not seen in the general population. Persistent infection has greater potential for causing precancerous lesions; the authors had no explanation regarding the continued high prevalence in the general population. The decline in prevalence with age was generally noted across regions[54] with a peak in the younger women as they become sexually active. The persistence of HPV in middle-aged women precludes once-in-a-lifetime cervical cancer screening strategies. This study, though limited in numbers points

to the chronic nature of infection and potential for cervical cancer to develop in later decades.

A consistent theme throughout this review is the unmet need for optimal cervical cancer screening. Yet this gap is also seen in access to treatment. Screening without treatment is unethical[104] and a waste of resources. Root cause analysis would focus on access to screening, continuity of care and treatment. Social determinants of inequality include many of the characteristics of the indigenous women in Guatemala: high rates of poverty, lack of education, extreme gender bias and rural residency, among the factors that make screening for cervical cancer difficult for Mayan women in Guatemala.

References

1. DiClemente RJ, Crosby RA, Kegler MC ed. *Emerging Theories in Health Promotion Practice and Research*. 2nd Ed. J Wiley & Sons, San Francisco, CA. 2009

2. Ogrodnik C, Borzutzky S. Women under attack: violence and poverty in Guatemala. *J of Inter Women's Studies*. 2011; 12: 55-67

3. Ruano S, Zambrano A; Guatemala: country gender profile; 2006, prepared for JICA – Japan International Cooperation Agency. https://www.jica.go.jp/english/our_work/thematic_issues/gender/background/pdf/e06gua.pdf. Accessed May 2, 2017

4. Reading Village. Guatemala. http://readingvillage.org/about-us/guatemala/ Accessed May 2, 2017

5. Guatemala Index Mundi *https://www.indexmundi.com/facts/indicators/SP.DYN.TFRT.IN/map/central-america* accessed May 3, 2018

6. Jensen KE, Schmiedel S, Norrild B, Frederiksen K, Ifter T, Kjaer. Parity as a co-factor for high –grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. *Br J Cancer* 2013; Jan 15; 108: 234-239

7. Munoz N, Franceschi S, Bosetti C, et al. Role of parity and human papillomavirus

in cervical cancer: the IARC multicentric case-control study. *The Lancet.* 2002; 359: 1093-1101

8. Chary A. Structural inequities in the hospital system: rural and indigenous women's experiences of cervical cancer treatment in Guatemala. *Revue Sociologic Sante* 2015; 38: 161-177

9. Moore, S, Forman D, Pineros M, Fernandez SM, de Oliveira Santos M, Bray F. Cancer in indigenous people in Latin America and the Caribbean: a review. *Cancer Medicine* 2014; 3: 70-80

10. Fonseca-Moutinho JA. Smoking and cervical cancer. *ISRN Obstet Gynecol* 2011; 2011 847684

11. Guatemalan Human Rights Commission/USA. For Women's Right to Live Program. http://www.ghrc usa/Programs/ForWomen'sRighttoLive/FAQs.htmlFFtop. Accessed May 2, 2017

12. Suarez J, Jordan M; Three thousand and counting: a report on violence against women in Guatemala, Guatemala Human Rights Commission, 2007 http://www.ghrc-usa.org/Programs/ForWomensRighttoLive/ThreethousandandCounting,AReportonViolenceAgainstWomeninGuatemala.pdf. Accessed May 2, 2017.

13. Woodward RL. *A Short History of Guatemala.* Antigua, Guatemala 2008

14. Forsythe, D. Ed. *Encyclopedia of human rights*, USA. Oxford University Press, 2009; 341.

15. Jonas S. *The Battle for Guatemala: rebels, death squads and US power*. Boulder, CO: Westover Press 1991.

16. Brankovic I, Verdonk P, Klinge I. Applying a gender lens on human papillomavirus infection: cervical cancer screening, HPV DNA testing and HPV vaccination. *Int J Equity Health.* 2013; 12:14.

17. Coker AL, Hopenhayn C, DeSimone CP, Bush HM, Crofford L. Violence against women raises risk of cervical cancer. *J Womens Health (Larchmt).* 2009; 18:1179-85.

18. Lawton AM. The right to health in indigenous Guatemala: prevailing historical structures in the context of health care. *Health and Human Rights J*. in Perspectives 8/27/2015 Retrieved from https://www.hhrjournal.org 2 May 2017

19. Ajzen I. From decisions to actions: A theory of planned behavior. In: *Kuhl J,*

Beckmann J, editors. *Action-control: From cognition to behavior*. Springer; New York: 1985. 11–39.

20. Ajzen I. *The Theory of Planned Behavior. Organizational Behavior and Human Decision Processes.*1991; 50: 179–211.

21. Albarracín D, Johnson BT, Fishbein M, Muellerleile PA. Theories of Reasoned Action and Planned Behavior as Models of Condom Use: A Meta-Analysis. *Psychological bulletin*. 2001; 127: 142-161.

22. Armitage CJ, Conner M. Efficacy of the theory of planned behavior: a meta-analytic review. *British J of Social Psych.* 2001; 40: 471-499.

23. Farmer P. *Social inequalities and emerging infectious diseases*. Emerging Infectious Diseases. 1996; 2: 259-269.

24. Bruni L,Barrionuevo-Rosas L, Albero G et al. *ICO Information Centre on HPV and Cancer (HPV Information Center). Human Papillomavirus and Related Diseases in Guatemala*. Summary Report 19 April 2017

25. Valles X, Murga GB, Hernandez G et al. High prevalence of human papillomavirus infection in the female population of Guatemala *Int J Cancer* 2009; 125: 1161-1167.

26. Roncancio AM, Ward K, Sanchez IA et al. Using the Theory of Planned Behavior to understand cervical cancer screening among Latinas. *Health Educ and Behavior.* 2015; 1-6.

27. Murillo R, Herrero R, Sierra M, Forman D. Cervical cancer control in Central and South America: Burden of disease and status of disease control. *Cancer Epidemiology.* 2016; 44 suppl 1: s121-130

28. Herrero R, Ferreccio C, Salmeron J et al. New Approaches to cervical cancer screening in Latin America and the Caribbean. *Vaccine* 2008; 26 Suppl 11: L49-L5

29. Joint United Nations Programme on AIDS (UNAIDS). Global AIDS Update 2016 http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016. Accessed May 2, 2017.

30. Meloni ST, Chang CA, Eisen G. et al. Long –term outcomes on antiretroviral therapy in a large scale-up program in Nigeria. *PLoS One* 2016;11:e0164030.

31. Partners in Health. Dr. Shyirambere: *Cancer is No Longer a Death Sentence in Rwanda.* http://www.pih.org/blog/dr.-cyprien-shyirambere-discusses-cancer-care-

in-rural-rwanda. Accessed May 2, 2017.

32. Rabkin M, El-Sadr WM. Why reinvent the wheel? Leveraging the lessons of HOV scale-up to confront non-communicable diseases. *Global Public Health* 2011; 6: 247-256

33. Winkler J, Bingham A, Coffey P, Handwerker WP. Women's participation in a cervical cancer-screening program in northern Peru. *Health Education Research* 2007; 23:1 10-24

34. Vasquez J Human Rights and Health: Indigenous Peoples; PAHO 2008. accessed 5/6/2017

35. Chary A, Rohloff P. ED, Privatization and the New Medical Pluralism, Shifting Healthcare Landscapes in Maya Guatemala; Lanham MD: Lexington Books; 2015

36. Green T, Green H, Scandlyn J, Kestler A. Perceptions of short term medical volunteer work: a qualitative study in Guatemala. *Globalization and Health* 2009 https://globalizationandhealth.biomedcentral.com/articles/10.1186/1744-8603-5-4 Accessed May 2, 2017.

37. Hospedales CJ, Samuels TA, Cummings R, Gollop G, Greene E. Raising the priority of chronic non-communicable diseases in the Caribbean. *Rev Panam Salud Publica* 2011; 30: 393-400

38. Nyar R, Wilber D. The pap test and Bethesda 2014. *ACTA Cytologica* 2015; 59:121-132 doi: 10.1159/000381842 accessed May 6, 2017

39. Burd E. Human papillomavirus and cervical cancer. *Clin Microbiol Rev* 2003; 16: 1-17

40. Gravitt PE, Winer RL. Natural history of HPV infection across the lifespan: Role of viral latency. *Viruses.* 2017; 9: 267; doi:0.3390/v9100267 accessed 4/17/2018

41. Schiffman M, Doorbar J, Wentzensen N et al. Carcinogenic human papillomavirus infection. *Nature Review/Disease Primer.* 2016; 2: 1-20

42. Koutsky L, Galloway D, Holmes K. Epidemiology of genital human papillomavirus infection. *Epidemiol Rev* 1988; 10: 122-163

43. Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. *Gynecol Oncol*. 2008; 110 (3 suppl 2): s4-7

44. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections

2005*; J Clini Virol;* 32 Suppl 2: S16-S24.

45. Weaver BA; Epidemiology and natural history of genital human papillomavirus infection. *J Am Osteopath Assoc* 2006; 106 (3 Suppl 1): S2-8.

46. Lie AK, Kristensen G. Human papillomavirus E6/E7 mRNA testing as a predictive marker of cervical carcinoma. *Expert Rev Mol Diagn.* 2008; 8:405-415.

47. Bosch FX, De Sanjose S. Human papillomavirus and cervical cancer burden and assessment of causality. J Natl Cancer Inst Monogr; 2003; 31: 3-13.

48. Chen AA, Heideman AM, Boon D et al. Human papillomavirus 45 genetic variation and cervical cancer risk worldwide. *J of Virol.* 2014; 8: 4514-21

49. Munger K, Baldwin A, Edwards KM et al. Mechanisms of human papillomavirus-induced oncogenesis. *J Virol*. 2004; 78: 11451-60.

50. Mirkovic J, Howitt BE, Roncarati P, et al. Carcinogenic HPV infection in the cervical squamocolumnar junction. *J Patho*. 2015; 236: 265-271.

51. Hamid NA, Brown C, Gaston K. The regulation of cell proliferation by the papillomavirus early proteins. *Cell Mol Life Sci*. 2009; 66: 1700-1717

52. Franco EL, Villa LL, Sobrinho JP et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection from a high-risk area for cervical cancer *J Infect Dis* 1999; 180:1415-23

53. Gravitt PE The unknowns of HPV natural history *J of Clin Invest* 2011; 121: 4593-99

54. Khan MJ, Castle PE, Lorincz AT et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Nat Cancer Inst* 2005; 97: 1072-1079

55. Drain PK, Holmes KK, Hughs JP, Koutsky LA. Determinants of cervical cancer rates in developing countries. *Int J Cancer* 2002; 100: 199-205

56. Burchell AN, Winer RL, de Sanjose S, Franco EL. Epidemiology and transmission dynamics of genital HPV infection. *Vaccine.* 2006; S3/52-S3/61

57. Gravitt PE, Rostitch AF, Silver M et al. A cohort effect of the sexual revolution may be masking an increase in Human papillomavirus detection at menopause in the United States. *J Infect Dis* 2013; 207: 272-280

58. Bruni L, Diaz M, Casrellsague X et al. Cervical Human papillomavirus prevalence in 5 continents: Meta-analysis of 1 million women with normal cytologic findings. *J Infect Dis* 2010; 202: 1789-1799

59. Houlihan CF, Baisley K, Bravo IG et al. Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania. *Intern J of Epi.* 2016; 762-773.

60. Plummer M, Peto J, Franceschi S. Time since first sexual intercourse and the risk of cervical cancer. *Int J Cancer* 2012; 130: 2638-44

61. Winer R, Hughes JP, Feng Q et al. Condom use and the risk of genital Human papillomavirus infection in young women. *N Engl J Med* 2006; 354: 2645-54

62. Winer R, Feng Q, Hughes JP, Kiviat NB, Koutsky LA. Risk of Human papillomavirus acquisition associated with first male partner. *J Infect Dis* 2008; 197: 279-82

63. Ansink AC, Cervical cancer in developing countries: how can we reduce the burden? Awareness raising, screening, treatment and palliation. *Trop Doc.* 2007; 37: 67-70.

64. Munoz N, Franco EL, Herrero R, Andrus JK, de Quadros C, Goldie SJ, Bosch FX Recommendations for cervical cancer prevention in Latin America and the Caribbean. *Vaccine.* 2008; 26 Suppl 11: L96-L107.

65. Lazcano-Ponce E, Alonso P, Ruiz-Moreno JA, Hernandez-Avila M. Recommendations for cervical cancer screening programs in developing countries. The need for equity and technological development. *Salud Publica Mex.* 2003; 45 suppl 3: S449-S462

66. Morse SA, Beck-Sague CM, Mardh PA (1999) Issues in the laboratory diagnosis of sexually transmitted diseases. In: K.K. Holmes, PA Mardh, P.F. Sparling, P.J. Wisner, Eds. Sexually Transmitted Diseases McGraw-Hill Book Co. New York, pp723-34.

67. Campos NG, Tsu V, Jeronimo J et al. When and how often to screen for cervical cancer in three low- and middle-income countries: A cost-effectiveness analysis. *Papillomavirus Res* 2015; 1: 38-58.

68. Denny L. Cervical cancer prevention: new opportunities primary and secondary prevention in the 21st century. *Int J Gynaecol Obstet*. 2012; 119: S80-4.

69. Jeronimo J, Morales O, Horna J, Pariona J, Manrique J, Rubiños J, Takahashi R. Visual inspection with acetic acid for cervical cancer screening outside of low-

resource settings *Rev Panam Salud Publica)* 2005;17: 1-5

70. Sherris, J, Wittet S, Kleine A et al. Evidence–based, alternative cervical cancer screening approaches in low-resource settings *Int Perspect Sex Reprod Health*. 2009; 35: 147-54.

71. Basu P, Meheus F, Chami Y et al. Management algorithms for cervical cancer screening and precancer treatment for resource–limited settings. *Int J Gynecol Obstet;* 2017; 138: 26-30

72. Fusco E, Padula F, Mancini E, Cavalier A, Grubisic G. History of colposcopy: a brief biography of Hinselmann. *J Prenatal Med* 2006; 2: 19-23

73. Denny L, Kuhn L, Pollack A, Wright TC. Direct visual inspection for cervical cancer screening: an analysis of factors influencing test performance. *Cancer* 2002; 94: 1699-1707

74. Gaffikin L, Lauterbach M, Blumenthal PD. Performance of visual inspection with acetic acid for cervical cancer screening: a qualitative summary of evidence to date. *Obstet and Gyne Survey* 2003; 58: 543-550

75. Luciani S, Winkler J. Cervical Cancer Prevention in Peru: lessons learned from the TATI Demonstration Project. Washington D.C.: PAHO 2006

76. Almonte M, Ferreccio C, Winkler J et al. Cervical screening by visual inspection, HPV testing, liquid-based and conventional cytology in Amazonian Peru. *Int J Cancer* 2007; 121: 796-802

77. Syrjanen K, Naud P, Derchain S et al. Comparing pap smear cytology, aided visual inspection, screening colposcopy, cervicography and HPV testing as optional screening tools in Latin America. Study design and baseline data of the LAMS study. *Anticancer Research* 2005; 25: 3469-3480

78. Sauvaget C, Fayette JM, Muwonge R, Wesley R, Sankaranarayanan R. Accuracy of visual inspection with acetic acid for cervical cancer screening. *Int J Gynec Obstet* 2010; 113: 14-24

79. Albert SO, Oguntayo OA, Samalla MOA. Comparative study of visual inspection of the cervix using acetic acid (VIA) and papanicolaou (pap) smears for cervical cancer screening. *Ecancermedicalscience* 2012; 6: 262 doi: 10.3332/ecancer.2012.262 accessed 12 April 2018

80. Choconte-Piraquive LA, Alvis-Guzman N, De la Hoz-Restrepo F. How protective is cervical cancer screening against cervical cancer mortality in developing countries? The Colombian case. *BMC Health Services Research.* 2010; 10: 270

81. Wright TC, Kuhn L. Alternative approaches to cervical cancer screening for developing countries. *Best Practice & Research Clinical Obstet and Gynec.* 2012; 26: 197-208

82. Blatt AJ, Kennedy R, Luff RD, Austin RM, Rabin DS. Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices. *Cancer Cytopathology* 2015; 123: 282-8

83. Fokom-Domgue J, Combescure C, Fokom-Defo V et al. Performance of alternative strategies for primary cervical cancer screening in sub-Saharan Africa: a systemic review and meta-analysis of diagnostic test accuracy studies. *BMJ* 2015; 351:h3084

84. Dawood R, El-Tahmoudy M. Visual inspection techniques versus pap smear in screening for premalignant and malignant lesions of the cervix in Menoufia governorate Egypt. *Tanta Medical J.* 2015; 43: 106-112

85. Mustafa RA, Santesso N, Khatib R et al. Systematic reviews and meta-analysis of the accuracy of HPV tests, visual inspection with acetic acid, cytology and colposcopy. *Int J of Gynec and Obstet.* 2016; 132: 259-265

86. Zhou H, Mody RR, Luna E, et al. Clinical performance of the Food and Drug Administration-approved high-risk HPV test for detection of high-grade cervicovaginal lesions. *Cancer Cytopathol* 2016; 124: 317-23

87. Raifa AO, El-Zein M, Sangwa-Lugoma G et al. Determinants of cervical cancer screening accuracy for visual inspection with acetic acid (VIA) and Lugol's Iodine (VILI) performed by nurse and doctor. *PLoS ONE* 12(1): e0170631. Doi: 10.137/journal.pone.0170631 accessed April 2018

88. Bishop A, Wells E, Sherris J, Tsu V, Crook B. Cervical cancer: evolving prevention strategies for developing countries. *Reproductive Health Matters* 1995; 3: 60-71

89. Palacio-Mejia LS, Rangel-Gomez G, Hernandez-Avila, Lanzcano-Ponce M. Cervical cancer, a disease of poverty: Mortality differences between urban and rural areas in Mexico. *Salud Public de Mex* 2003; 45 Suppl 3:s315-325

90. Maza M, Schocken CM, Bergman KL, Randall TC, Cremer M. Cervical precancer treatment in low- and middle–income countries: a technology review. *J Global Oncol* 2017; 3: 400-408

91. Schmidt C, Pretorius RG, Bonin M et al. Invasive cervical cancer following cryotherapy for cervical intraepithelial neoplasia or human papillomavirus

infection. *Obstet Gynecol* 1992; 80: 797-800

92. Chamot E, Kristensen S, Stringer JSA, Mwanahamuntu MH. Are treatments for cervical precancerous lesions in less-developed countries safe enough to promote scaling-up of cervical screening programs? A systematic review. *BMC Women's Health* 2010; 10:11

93. Ziyauddin F, Rajyashri SS. A study on the effect of cyrotherapy and LEEP in cervical dysplasia. *Biomedical Research* 2012; 23(4)

94. Jacob M, Broekhuizen FF, Castro W, Sellors J. Experience using cryotherapy for treatment of cervical precancerous lesions in low-resource settings. *Int J Gynec Obstet.* 2005; 89: 513-20

95. Catarino R, Petignat P, Dongui G, Vassilakos P. Cervical cancer screening in developing countries at a crossroad: Emerging technologies and policy choices. *World J Clin Oncol* 2015; 6: 281-290

96. Gottschlich A, Rivera-Andrade A, Grajeda E, et al. Acceptability of human papillomavirus self-sampling for cervical cancer screening in an indigenous community in Guatemala. *J Global Oncol.* 2017; 3: 444-54

97. Minority Rights Group International. *World Directory of Minorities and Indigenous Peoples- Guatemala: Maya* 2008: www.refworld.org/docid/497d163c.html accessed 2 May 2017

98. Sonnerburger PM. United Fruit Company In: ME Paige, Eds. *Colonialism: an international, social and political encyclopedia*. 2003 Santa Barbara CA

99. Adams WR, Rohloff P ed. *Health Care in Maya Guatemala.* University of Oklahoma Press*:* 2007 Norman OK

100. Maupin JN Fruit of the Accords: healthcare reform and civil participation in highland Guatemala. *Soc Sci Med*. 2009; 68: 1456-1463.

101. Burleigh E, Pro Redes Salud: Rapid Scale-up of Primary Healthcare through NGOs, Best practices in scaling up case study. John Snow Inc. 2007 Retrieved from www.jsi.com

102. Pena CL, Guatemala: improving access to health care services through the Expansion of Services Program (PEC): The case of Guatemala. Universal Health Coverage (UNICO) studies series; no. 19. Washington DC: World Bank 2013 retrieved from http://documents.worldbank.org/curated/en/2013/01/17210355.improving-access -health-care-services-through-expansion-coverage-program-pec-case-

guatemala. Accessed May 2, 2017.

103. Avila C, Bright R, Gutierrez J et al. *Guatemala Health System Assessment* 2015 Bethesda MD: Health Finance & Governance Project. Abt Associates, Inc.

104. Suba EJ, Murphy SK, Donnelly AD, et al. Systems analysis of real-world obstacles to successful cervical cancer prevention in developing countries. *Am J Public Health* 2006; Mar 96(3): 480-7

III: CERVICAL INFECTION WITH HUMAN PAPILLOMAVIRUS ANOGENITAL SUBTYPES IN INDIGENOUS WOMEN IN ALTA AND BAJA VERA PAZ, GUATEMALA

Anne Jeffries, CNM, MPH[1], Consuelo Beck-Sague, MD[1], Ariel Bernardo Marroquin Garcia[3],Virginia McCoy PhD[1], Purnima Madhivanan, MD, MPH, PhD[1,2], Eric Fenkl RN, PhD[1], Marta de la Cruz[3], Michael Dean, PhD[4]

[1]Florida International University, 11200 SW 8th Street, Miami, FL 33199;

[2] Public Health Research Institute of India, 89/B, 2nd Cross, 2nd Main, Yadavgiri, Mysore 560020, India

[3]Partner for Surgery, PO Box 388, McLean VA, 22101

[4]Laboratory of Experimental Immunology, National Cancer Institute Center for Cancer Research. National Institutes of Health, Frederick, Maryland.

Corresponding author:

Anne Jeffries, CNM, MPH

Department of Health Promotion and Disease Prevention,

Stempel College of Public Health and Social Work,

Room AHC 5 410, 11200 SW 8th Street,

Florida International University

Miami, FL 33199

ajeff004@fiu.edu

+1-561-252-8331

Abstract

OBJECTIVE:

Determine prevalence of cervical infection with oncogenic ("high-risk" [hr]) human papillomavirus (HPV) in indigenous women participating in two rural visual inspection with acetic acid (VIA) campaigns in 2013 and 2017, and assess the sensitivity and specificity of VIA in detecting hrHPV infection, and predictive values of normal and abnormal VIA for hrHPV infection.

METHODS:

Analysis of anonymous data collected during VIA clinics in 2013 (N=205) and 2017 (N=234) for indigenous women aged 21-65 years in six villages in Guatemala from whom specimens for hrHPV were collected at the time of VIA (total N=439).

RESULTS:

Of 393 with both VIA and hrHPV data, 89 (22.6%) had evidence of cervical hrHPV. hrHPV prevalence varied by year (29.6% in 2013, 17.4% in 2017; *P*=.004). Of these 89, 37 were reported with specific subtypes, of which 26 (70.3%) were HPV16, 1 (2.7%) was mixed HPV16/18, and 2 (5.4%) were HPV18. HPV16 was less common in 2013 (12/20 [60%]) than in 2017 (15/17 [88.2%]; *P*=.054). VIA was abnormal in only 10% of women with positive hrHPV subtype infection (sensitivity); specificity was 95.4%. Predictive values of positive and negative tests were 39.1% and 78.4%, respectively.

CONCLUSION:

These data suggest that exclusive VIA use may not detect many women with persistent infection and may be insufficient for screening. Real-time nucleic acid hrHPV detection may be superior, coupled onsite treatment and an effective HPV immunization program.

Introduction

Cervical cancer is one of the most studied and best-understood malignancies. Oncogenic ("high risk") human papillomavirus (hrHPV) subtypes are recovered in 95%-100% of cervical cancer specimens and have been established to cause most cases of cervical carcinoma[1-11]. Though cervical cancer is one of the most preventable and treatable cancers, it remains the fourth leading cause of cancer mortality in women worldwide[12]. Industrialized countries have reduced cervical cancer mortality through screening and over 80% of cervical cancer mortality currently occurs in low- and middle-income countries[13-18]. In 2010, cervical cancer was the fourteenth most frequent cause of cancer in US women and the mortality rate was 2.4/100,000[19].

In contrast, in low and middle-income countries, cervical cancer remains a leading cause of death despite the existence of effective and modestly priced screening, diagnosis and treatment. The Latin American and Caribbean regions are home to just nine percent of the world population, but bear approximately 16% of the world burden of cervical cancer mortality[14,16,17,20,21]. In Guatemala understanding hrHPV prevalence cervical cancer incidence and related mortality is difficult due to the low resources available to determine HPV prevalence and accurate data documenting incidence and mortality[7,8]. As a result, these have been imputed from surrounding countries, and from testing in specific areas.

Considerable differences in economic status and access to health care, present significant barriers to indigenous women in Guatemala. Over half (51%) of the population in Guatemala is documented as indigenous[22-24]; 90% of indigenous persons are classified as poor, or extremely poor[23,24]. Indigenous communities, particularly the large Maya population, endure much shorter life expectancies, 17 years shorter than non-indigenous groups[22-24] a consequence, of inaccessible health care, among many barriers for this population. Health expenditures in 2014 were 2.3347% of GDP in Guatemala[25], limiting health service expansion. In rural areas, there is a system for primary care consisting of 1,101 health posts employing a nurse, with varying access to mobile teams consisting of a physician, a nurse, and volunteers at the community level called "health facilitators" and "health guardians"[26-29].

To assess and address needs related to cervical cancer in underserved Guatemalan rural indigenous populations, visual inspection with acetic acid (VIA) has been proposed as it has in many underserved settings[9,31-35]. However, little is known of the prevalence of hrHPV oncogenic subtype infection in Guatemalan rural indigenous women, or effectiveness of VIA in identifying women with lesions suspicious for hrHPV , onsite "test-and-treat" strategies, and methods currently used to link them to specialized care[7,8,33]. To explore the prevalence of hrHPV subtype infection and the effectiveness of VIA in detecting hrHPV infection in this population, we analyzed anonymized data from women who were screened as part of a nonprofit organization's efforts to provide services to this population.

Methods

VIA Screening Clinics

The VIA screening clinics took place in two weeks in 2013 and 2017, respectively, in six villages, two of which were visited twice. These villages were located in Baja Vera Paz, immediately north of the capital region, and Alta Vera Paz, just north of Baja Vera Paz (Figure 5). The villages included Rabinal and Campur (each visited in both 2013 and 2017), Cahabon and Santa Cruz, visited just in 2013, and Carchar and Tactic, visited in 2017 only. The VIA screening clinics were supported by two NGOs that partner to bring services to rural Guatemala (Partners for Surgery and Timmy Global Health in 2013 and Partners for Surgery 2017). The schedule of the VIA clinic was coordinated with the local communities arranging for space to provide the clinic. The schedule and availability of screening was announced by Promotoras de Salud (female Community Health Promoters) employed by Partners for Surgery, who visited the areas where the clinics would be held two to four weeks before the event took place. Promotoras encouraged local women to participate in VIA screening. All women 21-65 years old denying pregnancy, presenting for and consenting to VIA testing were examined.

VIA Methodology and Specimen Collection

Guatemalan nurses who were undertaking the exam, both native Spanish speakers, obtained consent for the VIA screening. The nurses performing the exam had been trained during a governmental health department program and subsequently were hired by Partners for Surgery. Promotoras fluent in Q'eqchi and Mamean languages, the principal Mayan languages spoken in the region, translated for participants who were non-Spanish speakers. A separate consent for an additional sample for hrHPV nucleic acid testing used the form for a research study conducted by the National Cancer

Institute, National Institutes of Health (NIH). VIA was performed during a speculum exam to visualize the cervix just after collection of the specimen for hrHPV detection; a 3%-5% dilution of acetic acid (vinegar) was applied to the cervix, which was not a painful procedure. Visualization of the squamocolumnar junction in the cervix, where multi-layer tissue composed of squamous cells joins one-layer columnar tissue, was required for interpretation of VIA. Abnormal precancerous tissue and HPV-related lesions were expected to become white in appearance when exposed to vinegar, the principle on which VIA is based[35]. Specimen collection for HPV subtype determination with hrHPV testing was done prior to vinegar application during the same speculum exam with a Dacron swab.

High-Risk HPV Subtype Detection

The specimen was stored in a transport solution until shipment to the NIH laboratory without refrigeration, where hrHPV testing was done using the Hybrid Capture 2 assay (HC2; Qiagen, Germantown, MD) to detect hrHPV nucleic acid. This testing characterized HPV subtypes collected during the 2013 screening clinic in Rabinal, including hrHPV subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 with the kit, and additional subtypes detected in the NIH laboratory including subtypes 32, 67, 81, and 114. For samples collected in 2013 from other villages, hrHPV positive results were characterized as such without further subtype characterization. For samples collected during the 2017 screening clinics, Cobas HPV 4800 Amplification/Detection Kit (Roche Molecular Systems, Branchburg, NJ) to detect hrHPV including 16 and 18, and 12 pooled hrHPV subtypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). These were performed at the Palm Beach Pathology Laboratory (West Palm Beach, FL).

Treatment and Management of Women With Abnormal hrHPV and Results

Cryotherapy was used according to ministry of health guidelines for lesions believed to be benign but precancerous, but a Partner for Surgery staff member referred each woman for care in the case of an exam revealing physical changes suspicious for cervical cancer. The woman was advised regarding the disease, the urgency for treatment and the facilities that provide such care in the capital, Guatemala City, or local private care. Demographic data, VIA results and previous cervical screening history and results data were labeled with a patient identification number linked to the specimen identification for HPV subtype test, but was unlinked to any other patient identifiers. These de-identified anonymized data were forwarded to Florida International University investigators for analysis.

In the 2013 clinics, there was no protocol for advising Partners in Surgery staff about hrHPV results or for confirming that the women referred actually attended specialized care. In the 2017 clinic, cytology results were provided to Partners in Surgery staff so that Promotoras de Salud could advise the women with abnormal results of the need to seek specialized care, and where to seek such care for sliding scale or reduced prices.

Statistical Analysis

Data were entered into Excel files and analyzed using Epi Info for Windows Version 3.5.4 (Centers for Disease Control and Prevention, 2012, Atlanta, GA). Prevalence ratios (PRs) were used as estimates of relative risk when assessing the strength of associations; 95% confidence intervals (95%CIs) were used to assess precision of estimates and for statistical significance testing in bivariate analysis.

Medians were used as measures of central tendency for continuous variables, and the Kruskal-Wallis test for two groups, which has no assumption of normal distribution, was used for statistical significance testing. For analyses of categorical variables, the X^2 was used if all expected cells≥5; otherwise, Fisher exact two-tailed test was used. Sensitivity of VIA relative to hrHPV testing was calculated as the proportion of hrHPV positive tests for which VIA was abnormal, and specificity, as the proportion of hrHPV negative tests for which VIA was abnormal[36].

Results

Human Participant Ethical Review

The Florida International University Institutional Review Board reviewed the research and considered it a secondary analysis of de-identified data (not human participant research).

Population

Although 439 women consented to both tests, VIA results were available for only 413 women (189 in 2013 and 224 in 2017) and hrHPV results, only for 394 (170 in 2013 and 224 in 2017); results for both VIA and hrHPV results were available for 393. Most (76%) were aged 30 years or older (median=36 years; interquartile range [IQR]=30-43 years); 104 (62.3%) reported at least one prior cervical cancer cytological screening with a Papanicolaou ("pap smear") test. Just over half were interviewed in Mayan languages (over 80% of those in Q'eqchi). Almost half were interviewed in Spanish.

VIA and hrHPV Results

Of the women with both VIA and hrHPV subtype test results, 23 (5.9%; 95%CI=3.8%-8.8%) had abnormal VIA; 89 (22.6%; 95%CI=18.7%-27.2%) had nucleic

acid from at least one hrHPV subtype detected; 65.1% of women with hrHPV subtype infection were aged over 29 years, ranging from 30-58. Proportions of VIA exams that were abnormal did not differ significantly by year (7.2% in 2013, 4.9% in 2017), but hrHPV prevalence varied significantly by year (50 of 169 [29.6%] in 2013, 39 of 224 [17.4%], 2017; *P*=.004). Of these 89 with hrHPV, 37 were reported with specific subtypes, of which 26 (70.3%) were HPV16, 1 (2.7%) was mixed HPV16/18, and 2 (5.4%) were HPV18 (Figure 6). HPV16 was less common in 2013 (12/20 [60%]) than in 2017 (15/17 [88.2%]; *P*=.054).

Sensitivity of VIA for detection of hrHPV in 2013 and 2017 were almost identical at about only 10.0% (Table 2). The association between abnormal VIA and hrHPV detection was higher in 2017 than in 2013. In all combined, patients with abnormal VIA were about 1.8 times as likely to have hrHPV nucleic acid detected than patients who had normal VIA (*P*=.05). VIA specificity was 95.4%, with normal VIAs in over 95% of patients without hrHPV infection. VIA predictive positive and negative values for hrHPV were 39.1% and 78.4%. In the 2017 screening, one woman aged 47 years who had had cryotherapy three years before had a cervical tumor visible without magnification or acetic acid.

Discussion

Indigenous women in six rural villages in Guatemala had a moderately high prevalence of cervical hrHPV infection. Typing of subtypes among hrHPV-infected women was only done in about a third; distribution of subtypes differed somewhat by year. In 2017, using an assay that does not detect some of the subtypes reported in 2013, the prevalence was lower, with a higher proportion of infections due to HPV16 and 18

and lower proportion due to "pooled" subtypes. As a result, HPV 16, 18 and 31 contributed to relatively low proportions of infections. However, when restricted to results from villages for which hrHPV samples were typed, about 70% of infections were due to subtypes most likely to cause persistent infection and progress to cancer[16,36].

Untreated, many hrHPV infections spontaneously resolve, especially those in women less than 30 years of age[37], who usually are recently infected. However, almost two thirds of our participants with hrHPV cervical infection were over 29 years of age in our study. Conversely, HPV 16 is associated with more rapid progression and younger cancer onset[2]. Even among women with less aggressive subtypes, in this population, where marriage occurs early in life, and parity is high, hrHPV infections in young women may be more likely to represent persistent, progressive processes heralding malignancy.

Cyrotherapy has been largely abandoned in high-income countries because it has not been proven to completely remove abnormal tissue, and is considered of less value in treating abnormal cells within the cervical canal. Cryotherapy, without excluding cervical canal abnormalities – an area not evaluated during VIA – may not reduce risk for persistent infection. Cryotherapy can affect the transformation zone where chronic infection occurs, preventing adequate screening without colposcopy and endocervical canal biopsy and is of dubious value in post-menopausal women whose transformation zone migrates deeper in the os. Our data are insufficient to comment on the value of cryotherapy, which has been shown to increase regression to normal histology[38].

Limitations of this study include use of different assays for hrHPV detection, likely related to the narrower range of subtypes detected and lower proportion of tests positive in 2017. Conversely, lack of additional evidence of precancerous or cancerous

lesions in women tested in 2013 with hrHPV (because no tests, such as cytology, were done) leaves uncertain how many of these infections may have been clinically unimportant. Except for the one woman with cervical cancer after cryotherapy, no information was available on outcomes. Despite these limitations, our study suggests that in this underserved population, hrHPV-based test-and-treat, without VIA and even cytology, may be the most cost-effective strategy to reduce cervical cancer burden[39-41]. Coupled with scale-up of HPV immunization, hrHPV test-and-treat may reduce the toll that cervical cancer continues to exact in these underserved women.

References

1. Koutsky L, Galloway D, Holmes K. Epidemiology of genital human papillomavirus infection. Epi Review 1988;10:122-163

2. Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer Gyn Onc. 2008; 110 s4-s7.

3. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J of* Clinical Virology 32S. 2005; S16-S24

4. Haverkos H; Multifactorial etiology of cervical cancer: a hypothesis. MedGenMed. 2005; 7(4): 57.

5. Dailard C. The public health promise and potential pitfalls of the world's first cervical cancer vaccine. Guttmacher Policy Review. 2006; 9:6-9.

6. Thun M, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis. 2010; 31:100-110.

7. Bray F, Jermol A, Grey N, Forman D. Global transitions according to Human Development Index (2008-2030): a population-based study. Lancet. 2012; 13: 790-801

8. De Sanjose, S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010; 11:1048-1056.

9. Campos, NG, Tsu V, Jeronimo J, Mvundura M, Lee K, Kim JJ. When and how

often to screen for cervical cancer in three low- and middle-income countries: A cost-effectiveness analysis. Papillomavirus Research 1 2015; 38-58.

10. Burchell AN, Winer RL, de Sanjose S, Franco EL. Epidemiology and transmission dynamics of genital HPV infection. Vaccine. 2006 Aug 31; 24 Suppl 3:S3/52-6.

11. Burd E. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;

    16:1-17..

12. Stewart B, Wild, CP (eds.), International Agency for Research on Cancer, WHO. World Cancer Report 2014 [Online]. Available from: http://www.thehealthwell.info/node/725845 [Accessed: 17th May 2018].

13. Herrero R, Ferreccio C, Salmerón J, Almonte M, Sánchez GI, Lazcano-Ponce E, Jerónimo J. New Approaches to cervical cancer screening in Latin America and the Caribbean. Vaccine 2008; 26 Suppl 11:L49-58.

14. Arrossi, S, Ramos S. Social inequality in Pap smear coverage: identifying under-users of cervical cancer screening in Argentina. Reprod Health Matters. 2008; 16:50-8

15. Bosch FX, De Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J Natl Cancer Inst Monogr 2003; 31:3-13.

16. Clifford GM, Rana R, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14:1157-64.

17. Chamot E, Kristensen S, Stringer JSA, Mwanahamuntu M. Are treatments for cervical precancerous lesions in less-developed countries safe enough to promote scaling-up of cervical cancer screening programs? A systematic review; BMCWomen's Health 2010; 10:11.

18. Yano B, Bray F, Sellors JW, Zhang ZF. Cervical cancer as a priority for prevention in different world regions: an evaluation using years of life lost. Int J Cancer 2004; 109:418-424.

19. National Institute of Health. Cervical cancer fact sheet. Res Portf Online Report Tools. 2010; 1-2.

20. Campos NG, Tsu V, Jeronimo J et al. When and how often to screen for cervical cancer in three low- and middle-income countries: A cost-effectiveness analysis. Papillomavirus Research 2015; 1:38-58

21. Ansink AC. Cervical cancer in developing countries: how can we reduce the burden? Awareness raising, screening, treatment and palliation. Trop Doct 2007 37: 67-70.

22. Minority Rights Group International. World Directory of Minorities and Indigenous Peoples - Guatemala: Maya. World Directory of Minorities and Indigenous Peoples. http://www.refworld.org/docid/49749d163c.html. Accessed December 13, 2014.

23. World Bank. Guatemala: An Assessment of Poverty. Available at: http://go.worldbank.org/37PY9S2I50. Accessed January 2, 2016.

24. Vazquez J. *Human Rights and Health: Indigenous Peoples*. 2008. http://www.who.int/hhr/activities/indigenous_peoples/IndigPeople_10069.pdf.

25. The World Bank. Data Bank, Country: Guatemala, Current health expenditures as % of GDP. https://tradingeconomics.com/guatemala/health-expenditure-public-percent-of-gdp-wb-data.html accessed 5/18/18

26. Adams WR, Hawkins JP. Health Care in Maya Guatemala; University of Oklahoma Press: Norman; 2007.

27. Maupin JN. Fruit of the Accords: healthcare reform and civil participation in highland Guatemala. Soc Science and Med 2009; 68:1456-1463.

28. Burleigh E, Pro Redes Salud: Rapid Scale-up of Primary Healthcare through NGOs, Best practices in scaling up case study. Available at: https://www.jsi.com/JSIInternet/Inc/Common/_download_pub.cfm?id=10152&lid=3. Accessed May 17, 2017.

29. Pena CL. Guatemala: improving access to health care services through the Expansion of Services Program (PEC): The case of Guatemala. Universal Health Coverage (UNICO) studies series; no. 19. Washington DC: World Bank 2013 retrieved from http://documents.worldbank.org/curated/en/2013/01/17210355.improving-access - health-care-services-through-expansion-coverage-program-pec-case-guatemala.Accessed 5/2/17

30. Munoz N, Franco EL, Herrero R, Andrus JK, de Quadros C, Goldie SJ, Bosch FX Recommendations for cervical cancer prevention in Latin America and the Caribbean. Vaccine. 2008; 26 Suppl 11:L96-L107.

31. Murillo R, Altemonte M Pereira A, Ferrer E, Gamboa OA, Jeronimo J, Lazcano-Ponce E. Cervical cancer screening programs in Latin America and the Caribbean. Vaccine 2008; 26 Suppl 11 L37-L48.

32. Chary AN, Rohloff P, Major challenges to scale up visual inspection-based cervical cancer prevention programs: the experience in Guatemala. Glob Health Sci Pract. 2014; 2: 307-17.

33. Jeronimo J Morales O, Horna J, Pariona J, Manrique J, Rubiños J, Takahashi R. Visual inspection with acetic acid for cervical cancer screening outside of low-resource settings. Panam J of Public Health (Rev Panam Salud Publica) 2005;17:1-5

34. Sherris, J, Wittet S, Kleine A, Sellors J, Luciani S, Sankaranarayanan R, Barone MA. Evidence–based, alternative cervical cancer screening approaches in low-resource settings. Int Perspect Sex Reprod Health 2009; 35:147-54.

35. Carr KC, Sellors JW Cervical cancer screening in low resource settings: using visual inspection with acetic acid. J Midwifery Womens Health. 2004; 49:329-37.

36. Jaisamrarn U, Castellsagué X, Garland SM et al. HPV PATRICIA Study Group. Natural history of progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large, randomized PATRICIA study. PLoS One. 2013; 8:e79260.

37. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin. 2012; 62: 147–72.

38. Firnhaber C1, Swarts A, Goeieman B et al.. Cryotherapy Reduces Progression of Cervical Intraepithelial Neoplasia Grade 1 in South African HIV-Infected Women: A Randomized, Controlled Trial. J Acquir Immune Defic Syndr. 2017; 76:532-538.

39. Campos NG, Jeronimo J, Tsu V, Castle PE, Mvundura M, Kim JJ. The Cost-Effectiveness of Visual Triage of Human Papillomavirus-Positive Women in Three Low- and Middle-Income Countries Cancer Epidemiol Biomarkers Prev. 2017; 26:1500-1510.

40. Jin XW, Lipold L, Foucher J et al. Cost-Effectiveness of Primary HPV Testing, Cytology and Co-testing as Cervical Cancer Screening for Women Above Age 30 Years. J Gen Intern Med. 2016; 31:1338-1344.

41. LaMontagne DS, Bloem PJN, Brotherton JML et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009; 360:1385-94.

42. LaMontagne DS, Bloem PJN, Brotherton JML, Gallagher KE, Badiane O, Ndiaye C. Progress in HPV vaccination in low- and lower-middle-income countries. Int J Gynaecol Obstet. 2017; 138 Suppl 1:7-14.

Figure 5. Villages in which visual inspection with acetic acid screening clinics were held 2013 and 2017.

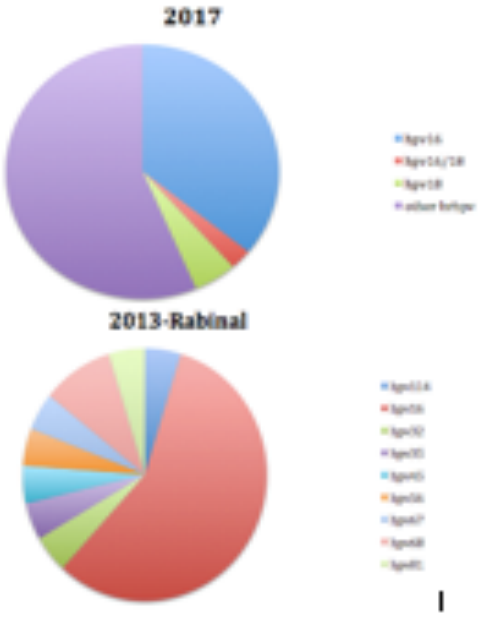


Figure 6. Distribution (proportions) of high-risk (HR) human papillomavirus (HPV) results by subtype, 2013 (50 of 169 specimens collected) and in 2017 (39 of 224 specimens collected), Alta and Baja Vera Paz, Guatemala.

NOTE: In 2013, subtypes were only reported on specimens from one village (Rabinal), with all positive tests from other villages characterized as "pooled" high-risk subtypes. In 2017, assay did not detect some subtypes, but HPV16 and HPV18 were reported from all villages.

Table 2

Sensitivity and Specificity of Visual Inspection With Acetic Acid (VIA) for Detection of hrHPV, Alta and Baja Vera Paz, Guatemala, 2013 and 2017, and 95% Confidence Intervals (95%CIs) and VIA Predictive Positive and Negative Values

Sensitivity, Specificity, Predictive Values of Positive and Negative VIA Tests

	2013	2017	Total
Sensitivity	5/50 (10.0%) 95%CI=3.3%-21.8%	4/39 (10.3%) 95%CI=2.9% 24.2%	9/89 (10.1%) 95% CI= 4.7% 18.3%
Specificity	112/119 (94.1%) 95%CI=88.3%-97.6%	178/185 (96.2%) 95%CI=92.4%-98.5%	290/304 (95.4%) 95%CI=92.2%-97.4%
Predictive Value of Positive	5/12 (41.7%)	4/11 (36.4%)	9/23 (39.1%)
Predictive Value of Negative	112/15 (71.3%)	178.213 (83.6%)	290/370 (78.4%)

Table 3

Association Between VIA and hrHPV Subtype Test Results in 2013, 2017 and Both Years Combined

	hrHPV Prevalence	Prevalence Ratio (95% CI)	*P*
2013 Abnormal VIA	5/12 (41.7%)	1.45 (0.71-2.97)	.42
2013 Normal VIA	45/157 (28.7%)		
2017 Abnormal VIA	4/11 (36.4%)	2.21 (0.96- 5.12)	.20
2017 Normal VIA	35/213 (16.4%)		
Total Abnormal VIA	9/23 (39.1%)	1.81 (1.05-3.1224)	.05
Total Normal VIA	80/370 (21.6%)		

IV: SENSITIVITY AND SPECIFICITY OF CERVICAL VISUAL INSPECTION WITH ACETIC ACID (VIA) COMPARED TO ONCOGENIC HUMAN PAPILLOMAVIRUS SCREENING IN RURAL INDIGENOUS GUATEMALAN WOMEN: TIME TO RETHINK VIA?

Anne Jeffries, CNM, MPH, PhDc[1], Consuelo M. Beck-Sagué[1], MD, FAAP, Ariel Bernardo Marroquin Garcia[2], Diego Aurelio Cordova Toma, RN[2], Virginia McCoy, PhD[1], Eric Fenkl, RN PhD[1], Purnima Madhivanan, MD, MPH, PhD[1,3]

[1]Florida International University, 11200 SW 8th Street, Miami, FL 33199;

[2]Partners for Surgery, PO Box 388, McLean VA, 22101;

[3] Public Health Research Institute of India, 89/B, 2nd Cross, 2nd Main, Yadavgiri, Mysore 560020, India

Address for corresponding author:

Anne Jeffries, CNM, MPH

Department of Health Promotion and Disease Prevention,

Stempel College of Public Health and Social Work,

Room AHC 5 410, 11200 SW 8th Street,

Florida International University

Miami, FL 33199

ajeff004@fiu.edu

+1-561-252-8331

Abstract

OBJECTIVE: This study compared the performance of visual inspection with acetic acid (VIA) within a screen-and-treat approach to oncogenic (high risk) human papillomavirus (hrHPV) testing relative to a "gold standard" of liquid-based thin-layer preparation for cytological cervical cancer screening examination in rural indigenous communities in Guatemala.

METHODS: During six days in September 2017, trained nurses screened 224 women aged 23-58 years for cervical cancer with VIA. Specimens for cytology and hrHPV subtype testing were obtained from the cervix prior to VIA with spatula and cytobrush, and placed immediately into PreservCyt, a methanol-based transport solution. Sensitivity of VIA and HPV subtype testing were evaluated as proportions of women with abnormal cytology (excluding abnormal squamous cells of undetermined significance) that had abnormal VIA or hrHPV subtype test results. Specificity was assessed as proportion of women with normal cytology that had normal VIA and no hrHPV subtypes.

RESULTS: Of 224 women tested, 221 had usable cytology specimens; of those, 10 (4.7%; 95% confidence interval [CI]=2.3%-8.5%) had abnormal results, including one carcinoma and four high-grade and five low-grade squamous intraepithelial lesions. Eleven of 224 (4.9%; 95%CI=2.5%-8.6%) had abnormal VIA and 39 (17.4%; 95%CI=12.6%-23.6%) had hrHPV subtypes detected. Sensitivity of VIA in identifying precancerous and cancerous lesions was 20.0% (95%CI=2.5%-55.6%); specificity was 96.1% (95%CI=92.4%-98.3%). Sensitivity and specificity of hrHPV-subtype testing in identifying precancerous and cancerous lesions were 100% (95%CI=71.7%-100%) and 88.7% (95%CI=83.9%-92.7%) respectively. A confirmed cancerous lesion was seen

using VIA in a patient who had had VIA and cryotherapy four years before. Positive and negative predictive values were 20.0% and 96.1%, and 30.3% and 100% for VIA and hrHPV testing respectively.

CONCLUSIONS: VIA-based screening may not consistently identify all women at highest risk for cervical cancer. hrHPV subtype test-and-treat strategies, usable in the field in low-resource settings, may be preferable to VIA-based testing.

Introduction

Cervical cancer incidence and mortality decline in high-income countries accompanied use of cytological screening[1] but effective screening strategies in low- and middle-income countries (LMICs) remain elusive. Oncogenic "high-risk" human papillomavirus (hrHPV) subtypes cause most cases of cervical cancer, one of the best-understood and preventable malignancies, and are present in 95%-100% of cervical cancer specimens[2]. Latin American and the Caribbean (LAC), home to just nine percent of the world population, bear approximately 16% of the world burden of cervical cancer mortality[3]. Lack of infrastructure for screening and treatment, particularly in remote rural populations, are important barriers to detection and treatment. Cervical cancer is believed to be the leading cause of cancer mortality among Guatemalan women aged 15-44 years[4].

In LMICs lacking consistent access to cervical cytology, including in LAC, visual inspection with acetic acid (VIA) with onsite cryotherapy have been widely adopted[5]. Significant challenges in training and implementation of VIA in Guatemala, accompanied by low reliability and acceptability, may have impacted public sector VIA screening coverage, which is estimated at 12% to 18%, augmented by non-governmental organizations to almost 40%[6,7]. VIA requires minimal equipment: acetic acid (diluted

vinegar, 3-5%), a light source and speculums to perform the exam. Although VIA does not allow examiners to "grade" lesions, examiners can treat visible acetowhite lesions with cryotherapy (ablation with liquid nitrogen) eliminating the need for multiple visits. The analysis described in this paper compared the performance of VIA to hrHPV subtype testing, relative to "gold standard" liquid-based thin-layer preparation cytology in rural indigenous communities in Guatemala.

Methods

Population

Women living in four rural majority indigenous communities (Rabinal, Tactic, Compur and Cahabon), villages in Alta and Baja Vera Paz, Guatemala, were invited to attend a VIA clinic at no cost by Partners for Surgery, a non-governmental organization which provides health services in rural villages including cervical cancer screening to women aged over 21 years. Local community health promotors employed by Partners for Surgery informed community members 14-21 days prior to the event.

Design

Cross-sectional analysis of de-identified data collected during brief interviews of participants, and by VIA, cytological examination and HPV testing.

Setting

Examinations took place in local health centers that allowed privacy for women to be interviewed assisted by bilingual Spanish-Mayan language interpreters, and examined by Guatemalan registered nurses trained in VIA. As women consented, a number was assigned to the cytology and hrHPV specimens and recorded on the VIA result form, to provide patients with results. Data were anonymized before release to researchers.

Liquid-Based Thin-Preparation Cytology and hrHPV Subtype Testing

Specimens for cytology and hrHPV subtype testing were obtained from the cervix prior to VIA with a spatula and cytobrush, and placed immediately into PreservCyt, a methanol-based transport solution. Cytology using Thin Prep (Quest Diagnostics, Alameda, CA) and hrHPV nucleic acid amplification using the Cobas HPV 4800 Amplification/Detection Kit (Roche Molecular Systems, Branchburg, NJ) to detect 14 hrHPV subtypes including 16, 18, 45, and 51 were performed at the Palm Beach Pathology Laboratory (West Palm Beach, FL). All results were provided to Partners for Surgery to distribute to patients. Women with abnormal cytology results were referred for diagnostic evaluation. Those with normal cytology and positive hrHPV testing were advised to have annual VIA.

VIA and Cryotherapy

A sterile disposable clear plastic vaginal speculum was inserted in the vagina and freshly prepared 4% acetic acid was applied to the cervix. After one minute, the cervix was inspected using a hand-held flashlight. If the squamocolumnar junction was visualized, VIA was considered adequate. VIA was considered positive when a well-defined, dense whitened area with regular margins was visible at the squamocolumnar junction or the transformation zone. It was considered negative if the squamocolumnar junction was visualized but no acetowhite lesions[5] were observed. Cryotherapy (ablation with liquid nitrogen) was offered to women with acetowhite lesions. Women whose VIA examination suggested malignancy (raised, irregular acetowhite lesion, bleeding on contact) were referred to specialized care on the day of examination with VIA results and directions to government facilities that offer subsidized care.

Data Analysis

Results from the liquid-based thin-layer preparation cytology were considered the "gold standard" to calculate sensitivity and specificity of VIA and hrHPV. Results with atypical squamous cells of unknown significance (ASCUS) were excluded from the analysis. VIA and hrHPV subtype test sensitivities were calculated as proportions of women with abnormal cytology specimens whose VIA or HPV test was abnormal or positive, respectively. Specificities for VIA and hrHPV testing were calculated as proportions of women with normal cytology specimens with normal VIA or hrHPV results. Prevalence of abnormal VIA exams and hrHPV tests among women with abnormal cytological examinations (excluding ASCUS with negative HPV) was compared to prevalence in women with normal cytological examinations. Prevalence ratios were used as estimates of relative risk; 95% confidence intervals (95%CIs) were used to assess precision of estimates, and along with Fisher exact two-tailed tests, for statistical significance testing. All analyses were performed with Epi Info v. 3.5.4 (Centers for Disease Control and Prevention, Atlanta, GA).

Human Participant Ethical Review

The Florida International University Institutional Review Board reviewed the research and considered it a secondary analysis of de-identified data (not human participant research).

Results

Nurses screened 224 women aged 23-58 (median age=36; interquartile range=30-43) years. Three (1.3%) had insufficient cells to perform liquid-based thin preparation cytological testing. Of the 221 with usable cytological specimens, 10 (4.7%; 95%CI: 2.3%-8.5%) had abnormalities, including a carcinoma in a woman who had had VIA and cryotherapy in 2015, and five low-grade and four high-grade intraepithelial lesions (excluding eight with ASCUS). Among those 10, seven had had at least one previous VIA examination. Eleven (4.9%; 95%CI: 2.5%-8.7%) of the VIA examinations were abnormal, including the carcinoma. Five of the 11 with abnormal VIA, including the one with the lesion, were referred to specialized care, and four cryotherapy treatments were administered; two others were asked to return for cryotherapy but did not do so. Fifty-eight (25.9%) women had never had a pelvic exam; median age of women without a prior pelvic exam was 33 years (vs. 38 years in others; p=0.001).

hrHPV subtypes were detected in 39 (17.4%; 95%CI: 12.7%-23.0%) women. hrHPV subtype testing detected all 10 (sensitivity=100%; 95%CI: 71.7%-100%) women with abnormal cytological examinations. VIA detected only 2 of 10 (sensitivity=20%; 95%CI: 2.5%-55.6%). Specificities of hrHPV subtype testing and VIA were 181/205 (88.7%; 95%CI: 83.9% 92.7%) and 194/202 (96.1%; 95%CI=92.4%-98.3%), respectively. Median ages and numbers of children of women with and without VIA abnormalities or hrHPV subtypes, did not differ significantly (data not shown).

Both abnormal VIA and positive hrHPV tests were associated with abnormal cytological results, but association with positive hrHPV tests was stronger and

statistically significant (Table 4). Positive and negative predictive values were 30.3% and 100% for hrHPV testing and 20.0% and 96.1% for VIA. Cryotherapy was used to treat four women with abnormal VIA who did not have abnormal cytology or hrHPV.

Discussion

In this small study, VIA and hrHPV subtype testing had very different sensitivities (20% vs. 100%) relative to cytology. Of the two women with abnormal VIA and cytological examinations, one had had an abnormal VIA and cryotherapy before, and presented with a cancerous lesion. These findings suggest that the sensitivity of VIA for cervical cancer screening conducted by highly trained nurses in this setting may be low compared to hrHPV testing, and that VIA-guided cryotherapy may not always affect the course of precancerous lesions. VIA may not be as reliable in menopausal women, and hrHPV testing in younger women, but in this study, age did not affect either. hrHPV prevalence in Guatemala sex workers at STI clinics and in women at community health centers was 67.3% and 38.1% respectively[8], higher than observed in our study population (17.0%), in which no variance was seen by age or parity, (which has been associated with higher persistence of hrHPV).

This study had several limitations. Because abnormal cytology prevalence was low (4.7%), precision of sensitivity and hrHPV positive predictive values were low. The use of cytological testing as a "gold standard" may underestimate hrHPV testing specificity, since hrHPV persistence may precede cytological evidence of precancerous changes. The study did not confirm abnormalities with biopsy, requiring ASCUS exclusion. The screening was offered in only four communities. Many women traveled hours by foot to attend, suggesting that women screened may have been more interested

in screening than the general rural Maya population, and may not reflect population-level risk.

Nevertheless, our study suggests that VIA-based screening may miss women with treatable precancerous lesions and points to the promise of point-of-care real-time nucleic acid hrHPV testing, concurring with the World Health Organization recommendation to use a strategy of 'screen with an HPV test and treat', over a strategy of 'screen with VIA and treat'[9]. In rural India, this approach decreased advanced cervical cancer and related deaths by over 40%, without significant reductions using cytological or VIA-based screening in comparison groups[10]. Cervical cancer is an important cause of death in Guatemala. Our findings suggest concerns with use of VIA alone for cervical cancer screening.

References

1. Johannesson G, Geirsson G, Day N. The effect of mass screening in Iceland, 1965–74, on the incidence and mortality of cervical carcinoma. *Int J Cancer*. 1978; 21:418–425.

2. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J of Clinical Virology* 2005; 32S:S16-S24

3. Arrossi S, Sankaranarayanan R, Parkin DM. Incidence and mortality of cervical cancer in Latin America. Salud Publica Mex. 2003; 45:5306-5314

4. Capote Negrin LG. Epidemiology of cervical cancer in Latin America. *eCancer Medical Science.* 2015; 9:577.

5. Herrero R, Ferreccio C, Salmeron J et al.. New approaches to cervical cancer screening in Latin American and the Caribbean. *Vaccine* 2008; 26 L49-586.

6. Chary AN, Rohloff P, Major challenges to scale up visual inspection-based cervical cancer prevention programs: the experience in Guatemala; *Glob Health Sci Pract* 2014; 2: 307-17

7. Bruni L, Barrionuevo-Rosas L, Alberro G et al. ICO Information Centre on HPV and Cancer (HPV Information Center) Human Papillomavirus and Related Diseases in Summary Report Posted 27 July 2017. http://www.hpvcentre.net/statistics/reports/GTM.pdf. Accessed 7/27/2017.

8. Valles X, Murga GB, Hernandaz G et al. High prevalence of human papillomavirus infection in the female population of Guatemala. *Int J Cancer.* 2009; 125: 1161-67

9. World Health Organization. WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. 2013

10. Sankaranarayanan R, Nene BM, Shastri SS et al. HPV screening for cervical cancer in rural India. *N Engl J Med.* 2009; 360: 1385-94

Table 4

Associations Between Abnormal Visual Inspection With Acetic Acid (VIA) and Cervical Cytological Results, and Between Oncogenic (High-Risk) Human Papillomavirus (hrHPV) Results and Cervical Cytological Results, Excluding Samples With Atypical Cells of Unknown Significance (ASCUS)

Screening Test Results for VIA and hrHPV Subtype Testing	Number (%) positive in women with abnormal cervical cytological results	Prevalence Ratio (95%CI)*	Fisher Exact 2-tailed p-value
VIA Results			
Abnormal	2/ 10 (20.0)	5.1 (1.2-21.0)	0.145
Normal	8/203 (3.9)		
hrHPV Test Results			
Positive	10/33 (30.3)	----†	<0.0001
Negative	0/180 (0)†	----	

*95% confidence interval; †Denominator=0 (no false positives); undefined, cannot divide by 0

V: FACTORS ASSOCIATED WITH CERVICAL INFECTION WITH HIGH-RISK HUMAN PAPILLOMAVIRUS ANOGENITAL SUBTYPES IN INDIGENOUS WOMEN IN ALTA AND BAJA VERA PAZ, GUATEMALA

Anne Jeffries, CNM, MPH[1], Consuelo Beck-Sague, MD[1], Purnima Madhivanan, MD, MPH, PhD[1,2], Virginia McCoy PhD[1], Eric Fenkl RN, PhD[1], Ariel Bernardo Marroquin Garcia[33], Michael Dean, PhD[4]

[1]Florida International University, 11200 SW 8th Street, Miami, FL 33199;

[2] Public Health Research Institute of India, 89/B, 2nd Cross, 2nd Main, Yadavgiri, Mysore 560020, India

[3]Partner for Surgery, PO Box 388, McLean VA, 22101

[4]Laboratory of Experimental Immunology, National Cancer Institute Center for Cancer Research. National Institutes of Health, Frederick, Maryland.

Corresponding author: Anne Jeffries CNM, MPH

Department of Health Promotion and Disease Prevention,

Stempel College of Public Health and Social Work,

Room AHC 5 410, 11200 SW 8th Street,

Florida International University

Miami, FL 33199

ajeff004@fiu.edu

+1-561-252-8331

Abstract

OBJECTIVE: To determine prevalence of cervical infection with oncogenic ("high-risk" [hr]) human papillomavirus (HPV) in indigenous women participating in two visual inspection with acetic acid (VIA) cervical cancer screening events and identify factors associated with hrHPV cervical infection in participants.

METHODS: De-identified Data collected by interview and assays during VIA cervical cancer screening events in 2013 (N=205) and 2017 (N=234) for indigenous women aged 21-65 years in six villages in Guatemala. Cervical specimens were collected for nucleic acid amplification testing to detect hrHPV before VIA was conducted.

RESULTS: Of 394 women with hrHPV results, 90 (22.8%) had nucleic acid from at least one hrHPV subtype detected, 48.3% of whom had infection with subtypes 16 or 18, the subtypes most likely to be associated with persistent infection and cancers. Women aged less than 29 years or reporting less than four pregnancies were more likely to have hrHPV cervical infection (36.8%, 27.3%, respectively) than those who were older or reported more pregnancies (18.7; <.0001 and 17.9%; P=.025, respectively). Women who reported use of progesterone injections or implants were more likely to have hrHPV detected (31.9%) than those who used other or no contraception (19.5%; *P*=.013). When controlled for other factors in logistic regression, younger age, lower parity, progesterone injection or implant use, or examination in 2013, were independently associated with hrHPV infection.

CONCLUSION: The association of hrHPV infection with long-acting progesterone contraceptive use may reflect progesterone's action as a co-factor promoting hrHPV persistence.

Introduction

Persistent cervical infection with several subtypes of human papillomavirus (HPV), (oncogenic or "high risk" [hr] HPV subtypes), have been recognized since the 1980s to be necessary for the development of cervical cancer[1]. However, infection alone with hrHPV subtypes does not appear to be sufficient to cause progression to cervical cancer. Several "cofactors" for the development of cervical cancer have been identified, including low literacy, multi-parity, and history of sexual abuse, older age, low socioeconomic status, and in some cases, racial and ethnic minority group status[2]. Screening tests that detect lesions before they progress to cancer, a process that for women with normal cell-mediated immunity, can take years or even decades, can arrest the process[1,2]. As such, many of the demographic and socioeconomic "cofactors" appear to reflect lower access to health care and preventive services, when controlled for hrHPV, cervical cancer screening and linkage to specialized care[2]. Lower access in Guatemalan indigenous women is related to language barriers, since many indigenous women do not speak Spanish, the national language, but rather, indigenous, mostly Mayan languages, and to very high rates of poverty (with over 70% of indigenous people, over half of Guatemala's population living in severe poverty)[3,4]. Disenfranchisement and marginalization of Guatemalan indigenous groups has coincided with much worse health outcomes than counterparts of European descent[5].

Biological factors have been investigated in other settings, particularly links to hormonal contraception, but their role in high-parity marginalized populations, believed to have less hormonal contraception exposure, remains less studied. The objective of this analysis was to estimate prevalence of cervical infection with hrHPV subtypes in indigenous women participating in visual inspection with acetic acid (VIA) cervical cancer screening activities in 2013 and 2017, and identify factors associated with hrHPV in participants.

Methods

VIA Screening Clinics

Six villages were visited in two weeks in 2013 and in 2017, including in Baja Vera Paz, north of the capital region, and Alta Vera Paz, north of Baja Vera Paz. Rabinal and Campur were each visited in both 2013 and 2017. Cahabon and Santa Cruz were visited only in 2013 and Canchar and Tactic, in 2017 only. The VIA screening clinics were supported by two NGOs that collaborate to bring services to rural Guatemala (Partner for Surgery and Timmy Global Health in 2013, Partner for Surgery in 2017). The VIA screening clinic schedule was coordinated with the local communities to arrange for space to provide the clinic. Availability of cervical cancer screening and time and date were announced by Promotoras de Salud (female Community Health Promoters) employed by Partners for Surgery, who visited the areas where the clinics would be held two to four weeks before the event. Promotoras encouraged local women to participate in VIA screening. All non-pregnant women 21-65 years of age who attended the events and consented to VIA were examined. Women had a brief interview to obtain basic

information (e.g., age, parity, previous cervical cancer screening and treatment, contraception).

VIA Methodology and Specimen Collection

Nurses who performed the exam obtained consent for the VIA screening. Promotoras fluent in the principal Mayan languages spoken in the region, translated for participants who were not Spanish speakers. A separate consent for an additional sample for HPV nucleic acid amplification testing used the form for a research study conducted by the National Cancer Institute, National Institutes of Health (NIH) in 2013. In 2017, a separate consent sought an additional sample for HPV nucleic acid amplification testing and for cytological testing. VIA was performed during a speculum exam to visualize the cervix just after collection of the specimen for hrHPV detection; a 3%-5% dilution of acetic acid (vinegar) was applied to the cervix. Visualization of the squamocolumnar junction in the cervix, where multi-layer tissue composed of squamous cells joins one-layer columnar tissue, was required for interpretation of VIA. Abnormal precancerous tissue and HPV-related lesions were expected to become white in appearance when exposed to vinegar, the principle on which VIA is based.[6] Specimen collection for HPV subtype determination in 2013 and 2017, and in 2017, cytology sample, were obtained prior to vinegar application during the same speculum exam with a Dacron swab.

High-Risk HPV Subtype Detection

In 2013, the specimen was stored in a transport solution until shipment to the NIH laboratory without refrigeration, where hrHPV testing used the Hybrid Capture 2 assay (HC2; Qiagen, Germantown, MD) to detect hrHPV nucleic acid. This testing characterized hrHPV subtypes 16, 18 and 11 “pooled” subtypes 31, 33, 35, 39, 45, 51, 52,

56, 58, 59, and 68; additional subtypes detected in the NIH laboratory included 32, 67, 81, and 114 for specimens collected in Rabinal in 2013. For other villages in 2013, no breakdown of hrHPV subtypes was provided. For samples collected in 2017, Cobas HPV 4800 Amplification/ Detection Kit (Roche Molecular Systems, Branchburg, NJ) was used to detect hrHPV including 16 and 18, and 12 pooled hrHPV subtypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) at the Palm Beach Pathology Laboratory (West Palm Beach, FL).

Treatment and Management of Women With Abnormal hrHPV and Results

Cryotherapy was used according to ministry of health guidelines for lesions believed to be benign but precancerous. Partner for Surgery staff members referred women for care in the case of an exam revealing physical changes suspicious for cervical cancer. Women were advised regarding the disease, the urgency for treatment and the facilities that provide such care in

the capital, Guatemala City, or local private care. Demographic and interview data, VIA results and previous cervical screening history were labeled with a patient identification number linked to the specimen identification for HPV subtype test (and cytology result in 2017), but were unlinked to any patient identifiers.

In the 2013 events, there was no protocol for advising Partners in Surgery staff about hrHPV results or for confirming that the women referred actually attended specialized care. In the 2017 events, cytology results were provided to Partners in Surgery staff so that Promotoras de Salud could advise the women with abnormal results of the need to seek specialized care, and where to seek such care for sliding scale or reduced prices.

Statistical Analysis

Data were entered into Excel files and analyzed using Epi Info for Windows Version 3.5.4 (Centers for Disease Control and Prevention, 2012, Atlanta, GA). Prevalence ratios (PRs) were used as estimates of relative risk when assessing the strength of associations between putative predictors and hrHPV infection; 95% confidence intervals (95%CIs) were used to assess precision of estimates and for statistical significance testing in bivariate analysis. The Kruskal-Wallis test for two groups, which has no assumption of normal distribution, was used for statistical significance testing for continuous variables. For statistical significance testing of categorical variables, the X^2 was used if all expected cells ≥ 5; otherwise, Fisher exact two-tailed test was used.

Human Participant Ethical Review

The Florida International University Institutional Review Board reviewed the research and considered the analysis of de-identified anonymized data forwarded to Florida International University investigators for analysis not human participant research.

Results

As was typical for this type of event, hundreds of women (N=205), and N=234 in 2017; total N=439) attended the VIA clinics. Many came on foot from villages miles away, and waited up to 7-8 hours for screening. hrHPV results were available from 170 women examined in 2013 in Campur, Rabinal, Cahabon and Santa Cruz, and 224 from women examined in 2017 in Campur, Rabinal, Carchar and Tactic (Total N=394, 89.4% of attendees); 90 (22.8%) had at least one hrHPV subtype detected. Of 60 positive hrHPV results from villages where specimens were characterized by subtype (Rabinal in both

years and Carchar and Tactic in 2017), 29 (48.3%) were subtypes 16 and 18, the subtypes most likely to be associated with persistent infection and cervical cancer. Women's ages ranged from 21 to 59 years (median=36 years; interquartile range [IQR]=30.0-43.0 years). Of 385 who gave information about contraceptive use (167 in 2013, 218 in 2017), most (234 [60.0%]) used some form of contraception. Data on language used during interview were available for 214 women of whom 48.1% were interviewed in Spanish. The proportion of women who reported no contraception was almost identical among women interviewed in Spanish (35.9%) and among those interviewed in Mayan languages (38.7%; *P*=.67). The most frequently reported contraception, used by 91 (23.5%), was long-acting progesterone injection or implant; 86 reported surgical sterilization and 28 (7.1%), oral contraceptives. Almost 67% reported at least one "Pap" cytological screening test in the past.

hrHPV prevalence did not vary significantly by village in either year, ranging from 26.3% in Rabinal to 36.8% in 2013, and from 17.1% in Rabinal to 17.9% in Canchar, but was significantly higher in 2013 (30.0%) than in 2017 (17.4%); P=.003 (Table 5). Women who were aged less than 29 years or who had had less than four pregnancies were also more likely to have hrHPV cervical infection. Women who reported progesterone injection or implant use were more likely to have hrHPV (31.9%) detected than those who used other or no contraception (19.5%; *P*=.013). When controlled for other factors in logistic regression, younger age, examination in2013, and progesterone implant or injection for contraception were independently associated with hrHPV co-infection (Table 6).

Discussion

VIA is often considered a cervical cancer screening option for women with little or no access to care. Yet the majority of women tested as part of the screening activities described here reported prior cytological cancer screening and contraception (mostly prescription or surgical sterilization) use. Since early as the 1960s, as the use of oral contraceptives increased worldwide, the effect of exogenous steroid hormonal contraception began to be investigated as a possible co-factor in cervical cancer development.[6] Studies focused on oral combined hormonal contraception, which was more widely used. Recently, long-acting reversible contraception delivered by implant, and less conveniently, by quarterly injection) has been promoted in LMICs where health care access may be difficult[7].

The association between hormonal contraception and cervical cancer has been increasingly investigated as cervical cancer cofactors with mixed results. In contrast, behavioral factors have lessened in importance as cofactors, since they have been noted to only modestly increase risk of development of cervical cancer in women testing positive for hrHPV[2]. Looking beyond circumstantial risk and into genetic factors both of the woman and of hrHPV itself, may help elucidate the role of exogenous hormone use. In 1990, Herrero *et al.*[8] demonstrated the association between long-term injectable progesterone contraception use and cervical cancer.

Several issues in this analysis limit its generalizability. Like many clinic populations, rural populations that overcome significant barriers to attend a VIA clinic, and report prescription contraception and prior cytological examinations, as the populations described in this report may not represent the most marginalized populations, but rather, highly motivated individuals who greatly value health care. The possibility of

recall and social desirability bias is present, as lapses (particularly failure to attend appointments for injectable progesterone) may be difficult to remember, and long-acting contraception is energetically promoted to marginalized populations.[7] The prevalence of hrHPV infection was high overall, but much higher in 2013. It is unclear why prevalence overall declined in 2017, although the higher number of attendees may reflect a change towards broader representation of lower-risk individuals.

Despite these limitations, this analysis demonstrates an association, independent of age and parity between long-term progesterone exposure and hrHPV infection, with higher risk in these users than in women who report not using any contraception and women using contraception other than progesterone-based methods. Essential to women's empowerment is fertility control, of particular importance in countries where women, particularly women of indigenous background, experience barriers, including discrimination[5], in healthcare access. The long-term delivery systems allow for less need to access health care and pharmacy, cost and greater privacy in their contraception use. Beyond the fertility control, progesterone-only products reduce or eliminate menstrual bleeding, decreasing monthly hygienic challenges and anemia.

Recent studies have been able to describe the natural history of hrHPV infections, evaluate genetic components of hrHPV subtype persistence, and the effect on the cervical tissue of hormones such as estrogen and progesterone[9,10]. The analysis of multi-parity associated with increased risk for cervical cancer has suggested that high hormonal milieu effect cervical tissue changes occurring after a birth which result in increased exposure of the transformation zone where hrHPV infection occurs[9,10]. Chagas et al.[9] in studying oral contraception and cervical cancer identified a gene polymorphism which, if

present, increases an oral contraceptive user's risk for cervical cancer. Additionally, it is known that estrogen and progesterone stimulate HPV16 expression producing anti-tumor suppression by E6 and E7 oncogenes.[9,10] Improved understanding of genetic interactions could help better define the risk for cervical cancer through specialized screening for women interested in use of hormonal contraceptives. Multiparity and hormonal contraception both increase women's hormone exposure, endogenous (hormonal support of pregnancy) and exogenous (contraception), both may increase risk for cervical cancer by promoting expression of hrHPV, underscoring the importance of accessible reproductive health care that includes cervical cancer screening for underserved women in Guatemala.

References

1. Koutsky L, Galloway D, Holmes K. Epidemiology of genital human papillomavirus infection. *Epidemiol Rev* 1988; 10: 122-163.

2. Schiffman M, Doorbar J, Wentzensen N, de Sanjosé S, Fakhry C, Monk BJ, Stanley MA, Franceschi S. Carcinogenic human papillomavirus infection. *Nature Review/Disease Primer* 2016;2:1-20

3. Organización Panamericana de la Salud/Organización Mundial de la salud (OPS/OMS) en Guatemala. Perfil de salud de los pueblos indígenas de Guatemala. Guatemala, 2016. (Pan-American Health Organization/World Health Organization in Guatemala. Health profile of indigenous populations in Guatemala. https://www.paho.org/gut/index.php?option=com_docman&view=download&category_slug=publications&alias=792-perfil-de-salud-de-los-pueblos-indigenas-de-guatemala&Itemid=255. Accessed May 30, 2018.

4. Hautecoeur M, Zunzunegui MV, Vissandjee B. Las barreras de acceso a los servicios de salud en la población indígena de Rabinal en Guatemala. *Salud Publica Mex.* 2007; 49: 86-93.

5. Chary A. Structural inequities in the hospital system: rural and indigenous women's experiences of cervical cancer treatment in Guatemala. *Revue Sociologic Sante* 2015; 38: 161-177.

6. Moreno V, Bosch FX, Muñoz N et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *Lancet.* 2002; 359: 1085-1092

7. Townsend JW, Sitruk-Ware R, Williams K, Askew I, Brill K. New strategies for providing hormonal contraception in developing countries. *Contraception.* 2011; 405: 405-409

8. Herrero R, Brinton LA, Reeves WC et al. Injectable contraceptives and risk of invasive cervical cancer: evidence of an association. *Int J Cancer.* 1990; 46: 5-7

9. Chagas BS, Gurgel APAD, Alves da Cruz et al. An interleukin-10 gene polymorphism associated with the development of cervical lesions in women infected with human papillomavirus and using oral contraceptives. *Infect Genet Evol.* 2013; 19:32-37

10. Timmons B, Atkins M, Mahendroo M. Cervical remodeling during pregnancy and parturition.*TrendsEndocrinol Metab.* 2010; 21: 353-361

Table 5

Characteristic Associated With Statistically Significant Increased Risk of High-Risk (hr) Human Papillomavirus (HPV) Cervical Infections in Rural Indigenous Women in Guatemala Attending Visual Inspection With Acetic Acid Cervical Cancer Screening Clinics

Characteristic	Number with hrHPV Infections/Total Number with Factor (%)	Prevalence Ratio (95% Confidence Interval)	*P*-value
Year, 2013	51/170 (30.0)	1.72 1.19-2.49	.003
Year, 2017	39/224 (17.4)		
Age less than 29 years	28/76 (36.8)	1.97 (1.35-2.86)	<.0001
Age 29 or older	59/315 (18.7)		
Less than 4 pregnancies	50/133 (27.3)	1.43 (1.05-2.23)	.0253
4 or more pregnancies	37/207 (17.9)		
Progesterone Injectable or Implant User	21/91 (31.9)	1.63 (1.12-2.38)	.013
Progesterone Injectable or Implant Non-User	58/297 (19.5)		

Table 6

Factors Associated With High-Risk Human Papillomavirus (hrHPV) Cervical Infection in Logistic Regression, Controlling for Examination in 2013, age Less Than 29 Years, Parity Less Than Four, and Progesterone Depot and Implant use, Confined to 387 Women Without Missing Values in all Variables

Factor	Odds Ratio	95% Confidence Interval	P-Value
Age less than 29 years	1.92	1.02-3.60	0.0421
Progesterone depot or implant use	1.80	1.04-2.12	0.0361
Parity less than four	1.32	0.76-2.30	0.3233
Examination in 2013	1.97	1.20-3.23	0.0077

VI: SUMMARY AND DISCUSSION

Summary

These analyses of data collected in the Vera Paz regions of Guatemala provide insight into the state of cervical cancer screening programs for underserved rural indigenous women in the region. As previously noted, the health system is reliant upon NGOs to extend services into the remote areas of the country[1]. The NGO that conducted VIA exams is tasked with screening, and aside from onsite, immediate cryotherapy, has limited avenues to provide or secure further treatment. In 2013, the evaluation of the VIA exam was a first look at hrHPV prevalence in the Vera Paz region. In 2009 Valles[2] reported there were no data available regarding the burden of high-risk (hr) human papillomavirus (HPV) infection in Guatemala, and the estimated coverage of cytological testing (with Papanicolaou tests ["pap smears"] or liquid preparations) was less than 10%. Since then, hrHPV prevalence in Guatemalan women in the general and sex worker populations have been reported as 38.1% and 67.3%, respectively[3]; 17% of indigenous women in Santiago de Atitlan, Guatemala, had at least one hrHPV subtype detected in self-obtained samples[4]. In evaluating prevalence of hrHPV in the region, six communities participated in the screening, with a combined prevalence of over 22.8%, somewhat higher than the estimated prevalence of 16.1% in Latin America and the Caribbean that Bruni[3] *et al.* reported.

An age-adjusted evaluation by Bruni[3] showed a peak in early adulthood, then a second peak in hrHPV infection in Central and South America at age 45, clearly emphasizing the need for lifelong screening. The small prevalence study in 2013 also

compared VIA to the hrHPV findings. Of the women examined during this activity only eight VIA exams were positive, and of the 42 positive hrHPV, only three of the women who tested positive had abnormal VIA exams. The 2013 study had unanswered questions. There was no comparison with cytology or pathology to confirm the presence of precancerous changes in chronic infections sought in the VIA exam.

Of importance is the setting for examination; the nurses were using hand-held flashlights with varying light devices and used vinegar stored in equipment cases in the transport vehicles. Adequate light is required to visualize the changes made by acetic acid application. Each site used for VIA exams had difficulty providing privacy and women had to present in the morning, some not being seen until late afternoon.[4] Heat exposure[5] could affect acetic acid composition, and cause burning and irritation.

In some studies, VIA sensitivity for high-grade cervical intraepithelial neoplasia (cancerous or very likely precancerous lesions) has ranged from over 50% to over 80%, varying by menopausal status[6,7]. However, VIA only detected 20% of cytological high and low-grade lesions during the 2017 screening activity. The screeners were dedicated Guatemalan nurses trained in VIA examination, traveling monthly to rural areas of Guatemala to provide screening. They work in difficult conditions, in clinics that often do not have running water, or adequate exam tables. In the 2017 study, 17.0% of women presenting for VIA had positive nucleic acid amplification tests hrHPV. VIA had a sensitivity of 20% in identifying abnormal cytological findings (excluding atypical cells of unknown significance). In contrast, sensitivity and specificity of hrHPV in detecting precancerous and cancerous lesions were 100% and 88.6% respectively. In a large recent study in Tanzania, VIA sensitivity relative to cytological and HPV testing was

unacceptably low, and varied significantly by screener experience, HIV infection status and age[8].

The VIA screening activities have the capacity to treat women with positive exams, onsite without requiring a return visit. During the 2017 VIA screening activity four women were treated with cryotherapy; all of the ones treated had normal cytology and negative hrHPV. Cryotherapy is recommended onsite to prevent losing women with positive tests to follow-up, in some LMICs as high as 80% of women with precancerous lesions do not receive treatment[9]. Missing the opportunity to treat prior to malignant disease substantially raises costs financially and in lives lost. Cervical cancer is a disease that affects women in the middle years of life; Yang et al.[10] calculated years of life lost (YLL) and found in Latin America and the Caribbean region, cervical cancer YLL represent a larger contribution than tuberculosis, maternal conditions or AIDS. However, none of the cryotherapy treatments were provided to women with evidence of precancerous lesions or persistent hrHPV infection.

The data suggest that the prevalence of hrHPV infection is high in this region of Guatemala, where access to screening programs is dependent upon mobile units supported by NGOs working independently or contracted by the Ministry of Health[1]. Poor indigenous women have high risk for cervical cancer primarily due to lack of screening resources, but also in the socio-demographic milieu of marginalization and isolation. Their high fertility rates have been established to contribute possible as a co-factor in risk in cervical cancer[11]. This region is one of the poorest in Guatemala, with limited road system; Owen[12] in 2009 analyzed Alta Vera Paz and found the total network consisted of 4.1% paved roads, 31.3% unpaved roads with 64.5% identified as trails. This

clearly demonstrates the remoteness of the area. Long identified barriers to cancer screening and treatment include rural residency, poverty and being uninsured[13,14] characteristics shared by many indigenous women in Guatemala in this region. In 2006 a qualitative study in Rabinal, Baja Vera Paz, one of the six communities participating in the VIA screening in 2013 and 2017, identified language and transportation as barriers to care, and noted that traditionally women first consult their husbands for their own health care and health care of the children[15]. These practices delay care and demonstrate the importance of education and community support of VIA screening. Empowering women by increasing their independent access to screening, and identifying and addressing beliefs that serve as a barrier to screening will help to provide acceptable screening in the community. Research has found the importance of involving the community in partnership for developing acceptable strategies[16] to involve women in screening and also understanding the recommendation for continued surveillance for life. Moreover, in general, women (particularly in crowded settings with low privacy), like indigenous women in Atitlan, Guatemala, prefer self-obtained noninvasive specimen collection[4]

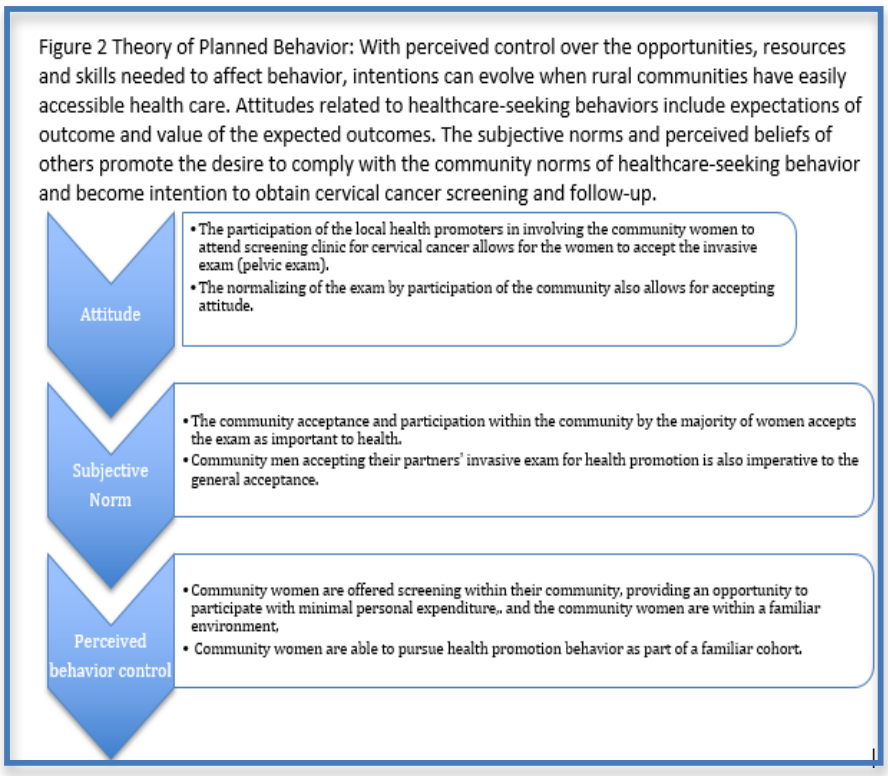
Screening will require more reliable strategies than VIA. VIA in this study fell short of expectations, but could be significantly enhanced or replaced with hrHPV subtype real-time testing onsite[17]. Self-sampling acceptability has been investigated in a small indigenous community in Guatemala and in other communities worldwide[4,18]. The authors determined that women preferred the self-sampling to an exam by physician, with 95% of the indigenous participants completing the study. Findings in the New Delhi study suggested that "self-HPV sampling compares favorably with physician-sampling and cytology. Rapid, affordable, HPV self-test kit can be used as the primary method

of cervical cancer screening in low-resource situations[18]. These studies offer a sampling method preferable to the pelvic exam in this indigenous community. Reserving VIA, pap and colposcopy for women identified as high risk due to hrHPV infection would conserve resources. A rapid, affordable, HPV self-test kit can be used as the primary method of cervical cancer screening in low-resource situations.

Theory of Planned Behavior

Some of the findings of the studies in this dissertation stand in contrast to the hypotheses, and even to the basic understanding of the cultural and social barriers to accessing women's health care, but they are amenable to explanation by the Theory of Planned Behavior. Although most women chose to be interviewed in their community's Mayan language, suggesting low acculturation, many of the cultural barriers assumed to occur in these communities were not identified. The majority of women accessing VIA reported previous cytological evaluation. Most (60.0%) reported contraceptive use. Clearly, their attendance at an event requiring hours of walking to arrive in the early morning and hours of waiting to be seen suggests that this group utilizes and values women's health services. In fact, the proportion of VIA clinic participants who reported modern contraceptive method use was substantially higher than the proportion of married women at the national level reporting modern contraceptive use in the population-based 2015 Guatemala Demographic and Health Survey (48.9%)[19]. It was also far higher than the proportion reported in a recent study of contraceptive use by women living in poverty in five Central American countries for Guatemala (27.5%)[20]. In that study, Guatemala's poorest women had the second lowest level of contraceptive use of the five countries

assessed, which ranged from 15.3% in Panama to 82.2% in Nicaragua. This same study identified indigenous ethnicity as a strong predictor of failure to use modern contraceptive methods.



In comparing the women attending the 2013 and 2017 VIA clinics to the women described in those reports, the Theory of Planned Behavior is instructive (Figure 2)**.** The work of community health promoters is the first step towards creating *attitudes* consistent with accepting cervical cancer screening. Their visible participation in recruitment, organizing and implementation of the activity normalizes the exam, and the numbers of women that attend each VIA clinic further reinforce the attitude that this is a valuable and desirable service. As dozens of neighbors of all ages attend the activities, the **subjective norm** shifts to acceptance by the community, including by male partners, of the need for

an intrusive examination to preserve health and prevent deaths of wives and mothers. Finally, the ownership of the activity by the community, familiarity of the setting, service availability at no cost, and presence of community translators and facilitators affirms women's *perceived behavior control*, in a setting of considerable peer support. This process, which begins with understanding screening and acceptance by the community allows women to seek screening for cervical cancer by intention[21] and participation in a community-screening event, has women supporting each other.

However, this activity is only as successful in prevention of cervical cancer as the efficacy of the screening method, and the linkage to effective care for women found to have precancerous or cancerous lesions. Identifying appropriate screening schedules and more reliable, cost-effective methods is a first step in adequate surveillance and improved estimates of cervical cancer incidence and mortality in indigenous populations and is vital to reducing the mortality associated with cervical cancer in this population. Screening ages have been evaluated for the greatest lifetime risk for cervical cancer reduction potential[22]. The natural history of hrHPV in immunocompetent women provides for intermittent screening, and the mean age of women with invasive cervical cancer is 50 years, as estimated by Baseman and Koutsky[23]. VIA is known to have decreased sensitivity in older[24,25] women, who at higher risk due to age, require a better screening tool and should be targets of screeners.

Guatemala struggles to provide cervical cancer-screening services to women; in 2008[26] it was estimated that only 40% of women had ever been screened. Self-HPV sampling compares favorably with physician sampling and cytology and clearly must be pursued. However, Chary followed women referred for specialized diagnostic testing and

treatment for cervical cancer and found that inadequate equipment, equipment in disrepair and understaffing constrained the system, reporting 1200-1700 patients on a waiting list for radiation therapy[27]. The lack of treatment options poses the question of health ethics: is screening and diagnosis in communities without access to treatment inconsistent with the aim of screening. The persistent determination is that infrastructure for screening and linkage to care is poor in many LMICs as in Guatemala. The strategy of utilizing a proven – not perfect – tool such as cytology may not be attainable at present for marginalized populations in LMICs but hrHPV testing may be.

Infrastructure development for cancer registries would help define the extent of the problem, as current incidence and mortality estimates are unreliable[28 29] particularly for indigenous women. Cervical cancer represents 36% of new cancers being treated in 2012[27] per Instituto de Cancerología (INCAN) patient registry, which also estimates only one third of patients initiating treatment complete therapy. Very often, the diagnosis of cervical cancer in members of marginalized populations is in later stages requiring specialized equipment, surgery and expensive chemotherapy or radiation therapy. This places considerable strain on the system as well as on families to little gain. Cervical cancer is predicted to increase by 75% in the region by 2025[24], threatening a crisis in indigenous women's survival.

Root cause analysis of cervical cancer screening problems would center the discussion on infrastructure, not only to screen, but also to include streamlining linkage from screening to definitive diagnosis to treatment to cure. Awareness of risk for cervical cancer and understanding of the natural history of hrHPV infection is paramount to community and individual adherence with screening recommendations, and should be

incorporated by the local health promoters working with NGOs. For indigenous women, language, illiteracy, poverty, discrimination and violence serve to marginalize them placing these women at higher risk for cervical cancer, the theory of Gender and Power is a descriptor of circumstance of many Guatemalan women.

Strengths and Limitations

The strength of these studies is that it was a glimpse at hrHPV prevalence in a previously understudied population. Valles[2] reported on prevalence comparing sex workers to "general population" (non-sex workers) women finding prevalence in women in the general population group did not decline with age as is expected; another small study in Atitlan indigenous women showed the acceptability of self-obtained samples.

Comparing VIA to cytology and hrHPV testing had not been done in Guatemalan indigenous populations prior to this study. VIA has been embraced by the Ministry of Health and therefore NGOs. While small, the study demonstrated a lower sensitivity than expected (22.2%) and a specificity of 96%. Conversely, with the strong showing of hrHPV testing (sensitivity and specificity: 100% and 88.6% respectively), real-time onsite hrHPV testing with or without VIA, reserving cytology and pathology for women with chronic hrHPV may save funds, time and staff resources, as a new infrastructure is established.

Limitations of the study include small sample sizes, with poor precision of estimates, low numbers of outcomes (e.g., one frank, invasive cancer after cryotherapy, nine low and high squamous cell) and confining population to Vera Paz, so that this study cannot be assumed to represent indigenous women in Guatemala, the largest such population in Central America. Clearly, the population's level of commitment to health

and prevention, use of contraception and cervical cancer screening makes them unrepresentative. The possibility of recall and social desirability bias when discussing these issues, where there is a clearly stated priority to engage indigenous women in these activities, is high.

Conversely, these women, clearly extremely poor, underserved, marginalized people, prove that community-based screening for cervical cancer can attract them, and obviously more than once. Even if these women exaggerate their use of contraception and cervical cancer screening, that exaggeration itself clearly reflects a level of normalization (Figure 7) that is essential for population-based uptake of screening.

Conclusions

For next steps in promotion of cervical cancer screening (and contraception), these communities can serve as "early adopters", partners in diffusion of innovation. Exploration of indigenous women's understanding of cervical cancer and their acceptance of treatment, particularly in absence of significant symptoms, is essential in developing measures to ensure adherence to screening and treatment recommendations. Building on Dr Chary's research on treatment at INCAN, following up with women referred for specialized diagnostic procedures and treatment from Vera Paz would help assess the effectiveness of cervical cancer screening.

References

1. Chary A, Rohloff P. ED, *Privatization and the New Medical Pluralism, Shifting Healthcare Landscapes in Maya Guatemala*; Lanham MD: Lexington Books; 2015

2. Valles X, Murga GB, Hernandez G, et al. High prevalence of human papillomavirus infection in the female population of Guatemala *Int J Cancer* 2009:125:1161-1167.

3. Gottschlich A, Rivera-Andrade A, Grajeda E, et al. Acceptability of human papillomavirus self-sampling for cervical cancer screening in an indigenous community in Guatemala. *J Global Oncol.* 2017; 3: 444-5

4. Bruni L, Diaz M, Casrellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical Human papillomavirus prevalence in 5 continents: Meta-analysis of 1 million woman with normal cytologic findings. *J Infect Dis* 2010: 202:1789-1799

5. New Jersey Department of Health: Harzardous substance Fact Sheet: Acetic Acid July 2007, revision March 2016

6. Poomtavorn Y, Suwannarurk K. Accuracy of visual inspection with acetic acid in detecting high grade cervical intraepithelial neoplasia in pre- and post-menopausal Thai women with minor cervical cytological abnormalities. *Asian Pac J Cancer Prev.* 2015; 16: 2327-31

7. Pothisuwan M, Pataradool K, Tangjigarmol S, et al. Visual inspection with acetic acid for detection of high grade lesion in atypical squamous cells and low grade squamous intraepithelial lesions from cervical pap smear. *J Gyencol Onc.* 2011; 22: 145-151

8. Dartell MA, Rasch V, Ifner T, et al. Performance of visual inspection with acetic acid and human papillomavirus testing in detection of high-grade cervical lesions in HIV positive and HIV negative Tanzanian women. *Int J Cancer.* 2014; 135: 896-904

9. Gage JC, Ferreccio C, Gonzales M et al. Follow-up care of women with abnormal cytology in a low-resource setting. *Cancer Detect Prev* 2003; 27: 466-471

10. Yang BH, Bray FI, Parkin DM, Sellors JW, Zhang ZF. Cervical cancer as a priority in different world regions: an evaluation using years of life lost. *Int J Cancer* 2004; 109: 418-424

11. Jensen KE, Schmiedel S, Norrild B, Frederiksen K, Ifter T, Kjaer. Parity as a co-factor for high –grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. *Br J Cancer* 2013, Jan 15; 108(1); 234-239

12. Owen KK, Obregon EJ, Jacobson KH. A geographic analysis of access to health services in rural Guatemala. *Int Health.*2010; 2: 143-9

13. Yabroff KR, Lawrence WF, King JC et al. Geographic disparities in cervical cancer mortality: what are the roles of risk factor prevalence, screening, and use of recommended treatment. *J Rural Health* 2005; 21: 149-57

14. Knaul FM, Gralow JR, Atun R et al. 2012; *Closing the cancer divide: overview and summary.* Boston: Harvard University Press

15. Hautecoeur M, Zunzunegui MV, Vissandjee B. Las barreras de acceso a los servicios de salud en la población indígena de Rabinal, Guatemala. *Salud Publica Mex* 2006; 49: 86-93

16. Agurto I, Arrossi S, White S et al. Involving communities in cervical cancer prevention programs. *Inter J Gynecol Obstet.* 2005; 89: 538-545

17. Sakaranarayanan R, Nene BM, Shastri SS et al. HPV screening for cervical cancer in rural India. *N Engl J Med.* 2009; 360:1385-94

18. Bhatla N, Dar L, Patro AR, et al. Can human papillomavirus DNA testing of self-collected vaginal samples compare with physician-collected cervical samples and cytology for cervical cancer screening in developing countries? *Cancer Epidemiol.* 2009; 33: 446-50.

19. Demographic and Health Surveys. Guatemala, 2014-2015. Indicator: Married Women Currently Using Modern Birth Control. StatCompiler. https://www.statcompiler.com/en/.

20. Rios-Zertuche D, Blanco LC, Zúñiga-Brenes P, Palmisano EB, Colombara DV, Mokdad AH, Iriarte E. Contraceptive knowledge and use among women living in the poorest areas of five Mesoamerican countries. Contraception. 2017;95:549-557.

21. Roncancio AM, Ward K, Sanchez IA et al. Using the Theory of Planned Behavior to understand cervical cancer screening among Latinas. *Health Educ and Behavior.* 2015: 1-6.

22. Campos NG, Tsu V, Jeronimo J, Mvundura M, Lee K, Kim JJ. When and how often to screen for cervical cancer in low- and middle-income countries: a cost

effective analysis. *Papillomavirus Res* 2015; 1: 38-58

23. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections 2005. *J Clini Virol;* 32 Suppl 2 S16-S24.

24. Cremer M, Conlisk E, Maza M et al. Adequacy of visual inspection with acetic acid in women of advancing age. *Int J Gynecol Obstet* 2011; 113: 68-71

25. Wright TC, Kuhn L. Alternative approaches to cervical cancer screening for developing countries. *Best Pract & Res Clin Obstet and Gynaec.* 2012: 26;197-208

26. Gakidou E, Nordhagen S, Obermeyer Z. Coverage of cervical cancer screening in 57 countries: low average levels and larger inequalities. *Plos Med.* 2008; 5: e132.

27. Chary A. Structural inequalities in the hospital system: rural and indigenous women's experiences of cervical cancer treatment in Guatemala. *Revue Sociologic Sante* 2015; 38, 161-177

28. Bruni L, Barrionuevo-Rosas L, Albero G, Mena M,Gomez D, Munoz J, Bosch FX, de Sanjose S. *ICO Information Centre onHPV and Cancer (HPV Information Center). Human Papillomavirus and Related Diseases in Guatemala*. Summary Report 19 April 2017 accessed May 11, 2017

29. Moore SP, Forman D, Pineros M et al. Cancer in indigenous people in Latin America and the Caribbean: a review. *Cancer Med.* 2014; 3: 70-80

VITA

ANNE JEFFRIES

Birthplace: Virginia, Minnesota

1979	Associate in Applied Sciences Marymount College of Virginia Arlington, Virginia
1981	Bachelor of Arts, Latin American Studies Newcomb College of Tulane University New Orleans, Louisiana
1984	Master of Public Health, Maternal/Child Health Tulane University, School of Public Health and Tropical Medicine New Orleans, Louisiana
1991	Practitioner Certification, Women's Health Services Education Program Associates San Jose State University Campbell, California
1996	Postgraduate Certificate in Nurse Midwifery University of Colorado, Health Sciences Center Denver, Colorado
2002 – Present	Certified Nurse Midwife Ob/Gyn Specialists of the Palm Beaches Jupiter, Florida
2014	Registered Nurse First Assistant (Surgery)
2018	Health Promotion and Disease Prevention Doctoral Candidate Florida International University Miami, Florida

PUBLICATIONS AND PRESENTATIONS

Jeffries, A., Beck-Sague, C., Marroquin Garcia, A., Cordova Toma, D., McCoy, H. V., Fenkl, E., and Madhivanan, P. (in press). *Sensitivity and*

Specificity of Cervical Visual Inspection with Acetic Acid Compared to Oncogenic Human Papillomavirus Screening in Rural Indigenous Guatemalan Women: Time to Rethink Visual Inspection with Acetic Acid? In Review. Sex Transm Inf (BMJ).

Jeffries A., Beck-Sague, C, Madhivanan, P., McCoy, H. V., Fenkl, E., de la Cruz, M., and Dean, M. (in press). *Factors Associated with Cervical Infection with High-Risk Human Papillomavirus Anogenital Subtypes in Indigenous Women in Alta and Baja Vera Paz, Guatemala.* For: Short Communication, Panam J Public Health (RPSP/PAJPH). Submission June 15, 2018 (pending NIH coauthor, Michael Dean, MD, PhD, sign-off).

Jeffries, A., Beck-Sague, C., Madhivanan, P., McCoy, H. V., Fenkl, E., de la Cruz, M., and Dean, M. (in press). *Anogenital High-Risk HPV Subtype Infection and Visual Inspection with Acetic Acid in Indigenous Women in the Vera Paz Regions of Guatemala*. For: Lessons from the Field, Bull WHO. Submission June 21, 2018 (pending NIH coauthor, Michael Dean, MD, PhD, sign-off).