Background

- India bears one-fifth of the global cervical cancer disease burden, largely a result of ineffective screening.
- Negative perceptions about visiting the doctor and compliance with pelvic examination when asymptomatic are barriers to screening programs in India.
- We are currently conducting the CATCH study, designed to compare the test characteristics of Pap, VIA, and HPV DNA screening methods under a typical rural Indian health infrastructure.
- In addition to the standard cervical sample, women were asked to provide a self-collected vaginal sample to evaluate the use of HPV-DNA testing on self-collected vaginal swabs as an alternative to clinic based screening.

Methods

Study design

This study is an interim analysis from a currently ongoing population based study (CATCH Study) of rural women in Medchal Mandal, Andhra Pradesh, India. CATCH Study eligibility – Age 25 years and older, not currently pregnant, not in labor, residing in Medchal Mandal, Andhra Pradesh, India. For this analysis, we tested paired cervical and vaginal samples from women enrolled as of January 31, 2006 with:

- a positive HPV DNA screen by Hybrid Capture-2 (h2C)
- a positive screening test by VIA or Pap smear
- women randomized to immediate colposcopy at the enrollment visit
- a random sample of remaining women

HPV DNA Detection

- All cervical and vaginal swabs were tested for presence of HPV 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, and 68 using Digene Hybrid Capture 2 (h2C) according to the manufacturer’s instructions. Test positivity was defined as 1.0 RU/LCO.
- We thank Digene Corp. for providing h2C kits at reduced cost for this project.
- All h2C positive samples, all women with a colposcopic exam, and a random sample of h2C-negative women without colposcopy were tested by PGM0101 consensus PCR and genotyped using the Roche prototype line blot (via kind donation from Roche Molecular Systems, Inc., Pleasanton, CA).

Overall, agreement of high risk and specific HPV results was estimated using kappa statistics. The Bayes formula was used for corrected sensitivity and specificity estimates for Pap, h2C, and VIA screening. The non-random sampling approach for PCR-based testing precluded our ability to generate corrected estimates.

Results

Table 1: Demographics of total CATCH Study population (N=841) and subset population (N=444)

<table>
<thead>
<tr>
<th>Total population (%)</th>
<th>Sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>240 (28.5)</td>
</tr>
<tr>
<td>30-39</td>
<td>155 (18.5)</td>
</tr>
<tr>
<td>40-49</td>
<td>65 (7.7)</td>
</tr>
<tr>
<td>50-62</td>
<td>101 (12.0)</td>
</tr>
<tr>
<td>Educated, self reported</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>286 (33.9)</td>
</tr>
<tr>
<td>Age at marriage (in years)</td>
<td></td>
</tr>
<tr>
<td>Don't Know</td>
<td>102 (12.3)</td>
</tr>
<tr>
<td>16-17</td>
<td>246 (29.1)</td>
</tr>
<tr>
<td>18+</td>
<td></td>
</tr>
<tr>
<td>Age at 1st pregnancy</td>
<td></td>
</tr>
<tr>
<td>Don't Know</td>
<td>95 (11.3)</td>
</tr>
<tr>
<td>16+</td>
<td>231 (27.5)</td>
</tr>
<tr>
<td>17+</td>
<td>160 (19.0)</td>
</tr>
<tr>
<td>Total</td>
<td>884</td>
</tr>
<tr>
<td>HPV-</td>
<td></td>
</tr>
<tr>
<td>HPV+</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>884</td>
</tr>
</tbody>
</table>

A sample included because sample was not/gyn negative.
†Consensus PCR positive for 1 or more of the 13 genotypes in the hc2 probe set (16, 18, 31, 33, 35, 45, 52, 56, 58, 59, 68).
§Includes 35 women with positive h2C screen who subsequently had a positive HPV DNA screen by Hybrid Capture-2.

The sample selected for these interim analyses was representative of the complete enrolled population as of January 2006.

Table 2: Agreement in HPV DNA detection: Hybrid-capture 2 (h2C) vs. consensus PCR in cervical samples

<table>
<thead>
<tr>
<th>HPV -</th>
<th>HPV +</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid Capture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV -</td>
<td>76</td>
<td>12</td>
</tr>
<tr>
<td>HPV +</td>
<td>12</td>
<td>335</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>347</td>
</tr>
</tbody>
</table>

- We confirmed the h2C test results with L1 consensus PCR, confirming the validity of local h2C testing in India.
- The overall agreement is 94.4% (κ = 0.8, McNemar’s p<0.01).
- Four HPV 16, 3 HPV 39, and one each of HPV 18, 52, 32, 33, 59, and 31 were ‘missed’ by h2C.
- Of the h2C-PCR negative samples, 3 were PCR positive with low risk types known to cross-react with h2C high risk probe type (e.g., HPV 53 or 66); and 8 had an L1 consensus PCR = 0.0 reflecting low viral load.

HPV Genotype Distribution

- In the general screening population, HPV 16 was the most commonly reported genotype, followed by HPV 32, 31, and 42.
- Among the eight women with PCR-positive CIN 2+ results, 6 were single HPV 16 infections, 3 were HPV 16 positive plus co-infection with HPV 18, 82, and 52, respectively, and one was an HPV 51 single infection.

Sensitivity and Specificity Estimates

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncorrected</td>
<td>Corrected</td>
<td>Uncorrected</td>
</tr>
<tr>
<td>VIA</td>
<td>22.2</td>
<td>9.8</td>
</tr>
<tr>
<td>h2C</td>
<td>77.8</td>
<td>61.9</td>
</tr>
<tr>
<td>HR-PCR, cervical</td>
<td>88.5</td>
<td>*</td>
</tr>
<tr>
<td>HR-PCR, vaginal</td>
<td>77.8</td>
<td>*</td>
</tr>
</tbody>
</table>

- The prevalence of CIN 2+ in this interim analysis is 9/841 (1.1%).
- While results should be interpreted with caution because of the limited power in this interim analysis, our data suggest that HPV testing from a self-collected vaginal swab would yield comparable test performance to cervical HPV testing, and superior performance to Pap or VIA-based approaches.

Conclusions

- We observed excellent agreement between hybrid capture-2 and consensus PCR HPV DNA detection in cervical samples, confirming inter-laboratory concordance between Johns Hopkins Bloomberg School of Public Health and Center for DNA Fingerprinting and Diagnostics.
- We also observed good agreement between cervical and vaginal HPV DNA test result, suggesting that vaginal samples offer a feasible alternative to HPV DNA testing in rural India.
- The fact that all women enrolled into the CATCH study as of January 31, 2006 consented to administering a self-visual sample suggests that self-sampling would be an attractive alternative to clinic-based cervical exams. This is an important advance as our formative research has identified the speculum-assisted pelvic exam as a common barrier to cervical cancer screening participation in India.
- Programmatic development research should investigate the feasibility of village-based self-sampling collection to determine if this alternative could offer a practical means of broad coverage cervical cancer screening in rural India.
- Use of newly developed rapid HPV tests in a field-based screen and treat scenario might be a practical alternative to cervical cancer screening in rural India.
Feasibility of field-based self sampling of adult women in Andhra Pradesh: Pilot study results from the CATCH Study


1Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, 2Center for DNA Fingerprinting and Diagnostics, Hyderabad, IN, 3SHARE India, Hyderabad, IN

Background

India bears one-fifth of the global cervical cancer disease burden, largely as a result of ineffective screening.

- Negative perceptions about visiting the doctor and compliance with pelvic examination when asymptomatic are barriers to screening programs in India.

- Primary screening that avoids pelvic exams and clinic visit would likely be an attractive option in rural India, The CATCH Study is an on-going population based cervical cancer screening study in rural Andhra Pradesh, India.

- We observed good agreement between self-collected vaginal and physician-collected cervical samples which suggests that vaginal swabs are a feasible alternative sampling.

- Agreement of vaginal and cervical PCR-based HPV detection was 91.5% (95% CI: 88.5, 94.5) and 90.1% (95% CI: 87.5, 92.6) for self vs. clinic collection, respectively. Studies targeting self sampling in rural India should test these tools on the population that will use them, focusing on acceptability, feasibility, and ability to access the samples.

- The objective of this study is to determine the feasibility of using field-based self collected vaginal sampling in the CATCH Study.

Study Design

- **CATCH Study design**
  - Age 25 years and older
  - Not currently pregnant
  - Intact uterus
  - Residing in Medchal Mandal, AP, India

- **Study participant**
  - Completes interview-administered questionnaire
  - Provides serum and self-collect vaginal swab
  - Gynecologist administered VIA, Pap smear and HPV DNA testing at a local hospital

Follow-up

For this analysis (as of October 1, 2007) —
- In 15 villages: All women who previously participated in CATCH Study (N=536; median age ≥30 years)
- In 9 villages: Eligible women who previously refused to participate in CATCH (N=388)

- Prior to field based self sampling program implementation, 3 FGDs were conducted among CATCH Study participants and non-participants to access the acceptance of a field-based self collected HPV program.

- Eligible women are contacted by field staff (2 Health supervisors (HS) & 1 counselor) in a systematic house-to-house strategy.

- Willing women who previously refused in CATCH provide consent to enroll and are asked to complete an interview administered questionnaire.

- All willing women are provided with the conical brush from a Digene sampler kit. HS verbally instructs women how to collect the self vaginal swab.

- Women were a part of their area and collected the sample using the brush and placed the brush in the collection vial with the HS. The HS labels the vial with the appropriate barcode label.

- All cervical and vaginal swab samples were tested for presence of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 using Digene world wide (ww) PCR (hc6) according to the manufacturer’s instructions. Test positivity was defined as ≥1 RLU/CO.

- Statistical Methods
  - Pearson’s chi-squared and z-tests were used to determine difference in participation rates
Background

- India bears one-fifth of the global cervical cancer disease burden.
- Evidence of effective screening programs is seen in developed countries to reduce the burden and impact of cervical cancer.
- Negative perceptions about visiting the doctor when asymptomatic are barriers to screening programs in India.
- Compliance with cervical examination is a predominant barrier for screening programs in India.
- We seek to evaluate the use of HPV-DNA testing on self-collected vaginal swabs as an alternative to clinic based screening.

The REACH Model

REACH is an experiment in alternate strategy, which has four critical elements:
- Extensive and intensive use of Information Technology (IT) including detailed census and household enumeration of the village population.
- Well equipped and well staffed rural hospital.
- Mobility of doctors and patients – doctors attend village clinic and provide transportation of patients to local hospital when needed.
- Village Clinics and Community Health Volunteers (CHV’s).

CATCH Study Design

Methods: Pilot study

Study eligibility –
- Age 30 years and older
- Not currently pregnant
- Intact uterus

All age-eligible women were identified from the census database. Health supervisors and community health volunteers (CHVs) went house-to-house evaluating further eligibility and personally inviting eligible women to participate. Consenting women were brought to the rural hospital in groups of 10-20 for enrollment.

Enrollment

The following was collected at enrollment:
- Written informed consent
- Questionnaire (demographics, reproductive history, contraceptive history, tobacco use, medical history)
- Blood (10 ml, serum)
- Self-collected vaginal swab (Digene Sampler Kit)
- Pelvic exam
- Pap smear (Ayer’s spatula and endocervical brush fixed in 95% EtOH)
- Cervical-collected cervical swab (Digene Sampler Kit)
- HPV testing by Hybrid Capture 2, high risk probe pool
- VIA performed by gynecologist (training at IARC study site).
- Vaginal self-collected swab (diagnostic swab)
- Health education through audiovisual aids.
- ALL DATA CENTRALLY MANAGED WITH INFORMATION TECHNOLOGY GROUP

Follow-up

- Results hand-delivered to participants
- Women with any positive screening test asked to return for colposcopy.

Reasons for non-participation

No reason reported 164 (54%)
Screening not important 28 (20%)
No time, inconvenient 21 (15%)
Fear a cancer diagnosis 19 (12%)
Confidentiality concerns 14 (10%)
Distrust of doctors 9 (2%)
Child care needs 2 (1%)
Old age 1 (1%)
Other 53 (38%)

Focus Group Discussions

We conducted focus group discussions among women aged 30-50 who had and had not participated in our pilot study to obtain a more detailed understanding of the barriers to study participation.

Results

Demographics of enrolled population

<table>
<thead>
<tr>
<th>Age</th>
<th>No</th>
<th>Yes</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-35</td>
<td>45</td>
<td>162</td>
<td>29.5</td>
</tr>
<tr>
<td>36-40</td>
<td>49</td>
<td>175</td>
<td>29.0</td>
</tr>
<tr>
<td>41-45</td>
<td>44</td>
<td>161</td>
<td>25.7</td>
</tr>
<tr>
<td>46-50</td>
<td>56</td>
<td>194</td>
<td>29.6</td>
</tr>
<tr>
<td>Ever attend school?</td>
<td>134</td>
<td>78</td>
<td>57.0</td>
</tr>
<tr>
<td>Indo side toilet?</td>
<td>40</td>
<td>150</td>
<td>37.5</td>
</tr>
<tr>
<td>Age at marriage (years)</td>
<td>14-16</td>
<td>101</td>
<td>55.2</td>
</tr>
<tr>
<td>17+</td>
<td>42</td>
<td>21.1</td>
<td></td>
</tr>
<tr>
<td>Pre-marital status</td>
<td>136</td>
<td>72.7</td>
<td></td>
</tr>
<tr>
<td>Separated</td>
<td>52</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>1</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Age at 1st pregnancy (years)</td>
<td>16-19</td>
<td>15</td>
<td>57.1</td>
</tr>
<tr>
<td>20-24</td>
<td>19</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Tubal ligation</td>
<td>18</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Ever used birth control?</td>
<td>36</td>
<td>150</td>
<td>53.6</td>
</tr>
<tr>
<td>Age at tubal ligation (years)</td>
<td>17</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>19-24</td>
<td>174</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>25+</td>
<td>50</td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td>Can’t remember</td>
<td>20</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Ever had a Pap smear?</td>
<td>148</td>
<td>79.6</td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td>18</td>
<td>12.1</td>
<td></td>
</tr>
</tbody>
</table>

| Total | 489 | 489 | 100.0 |

Table 3. Age-stratified screening prevalence

<table>
<thead>
<tr>
<th>AGE</th>
<th>VIA</th>
<th>PAP</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-35</td>
<td>45</td>
<td>6 (13.3)</td>
<td>6 (13.3)</td>
</tr>
<tr>
<td>36-40</td>
<td>46</td>
<td>2 (4.4)</td>
<td>7 (15.2)</td>
</tr>
<tr>
<td>41-45</td>
<td>35</td>
<td>2 (5.6)</td>
<td>6 (16.7)</td>
</tr>
<tr>
<td>46+</td>
<td>51</td>
<td>0 (0.0)</td>
<td>24 (47.1)</td>
</tr>
</tbody>
</table>

Conclusions

- Women were generally reluctant to participate in cervical cancer screening programs despite provision of free services and transportation. Results from refusal questionnaire and focus group discussion data suggest that a lack of understanding of preventive screening is largely responsible. The design of a simple educational message that would convey the concept of ‘pre-cancer’ and early detection and treatment would likely be of great value in relatively poorly educated areas of rural India.
- The high risk for cervical cancer in the rural Indian population was confirmed by observation of low rates of previous screening, 10% prevalence of high-risk HPV among women at least 7 years past first sexual experience, and high parity. Preliminary data suggests age-specific differences in test performance, particularly with false positive VIA among young women and false positive Pap among older women.
- In the REACH model of combined hospital and community-based health care delivery to the rural Indian population, we were able to implement all of the leading candidate screening technologies: VIA, HPV DNA testing, and Pap smears. Continuation of the CATCH project will allow a direction comparison of testing performance of each assay in the rural Indian environment as described.
- Based on the results of this pilot study, our primary study design has incorporated the following changes: decreased age eligibility to 25 years and older, immediate randomization of 20% of women to colposcopy at the enrollment visit, and community-wide education and awareness campaign guided by qualitative research in the community (e.g., focus group discussions, etc.). This includes community education by trained health counselors and development of an educational film (fictional drama) to stress the importance of early detection and treatment in the absence of visible symptoms.

We would like to acknowledge funding support from the International Agency for Research on Cancer (IARC, Lyon), an NCEO-US collaborative grant from the Department of Biotechnology, Ministry of Science and Technology, Government of India and the NIH, USA (5T32ES007063-14), and an NIH SPORE grant (P50 CA92522). We would also like to thank Digne Diagnostics for competitive pricing of hct kits, and Roche Molecular Systems for the donation of PCR reagents.
Seroepidemiology of HPV 11, 16, and 51 in a population-based sample of adult women in rural India

Gravitt P1*, Paul P1, Sowjanya P1, Ramkrishna G1, Ratnakar N1, Bahwani K1, Jain M1, Das M1, Reddy P1, Vijayraghavan K1, Shah KV2, Viscido R1.

1 Johns Hopkins University, 2 Center for DNA Fingerprinting and Diagnostics, 3 MedCiti Institute of Medical Science, SHARE INDIA

Introduction

• There is a lack of data to describe the burden of HPV type-specific infection among women in rural India, who have a disproportionately high rate of invasive cervical cancer.

• We estimated the cumulative lifetime exposure burden to HPV types 11, 16, and 51 in the first 841 married adult women from rural Andhra Pradesh, India enrolled in our ongoing HPV screening study (beginning January 2005) – the CATCH study.

CATCH Study Design

HPV SEROLOGY

• HPV 16 serostatus was determined by use of an HPV 16 Virus-Like Particle (VLP) ELISA.


• Cut points for seropositivity were defined as OD values five standard deviations above the mean value obtained from negative control sera referenced to seropositivity of children.

DNA DETECTION

• All cervical swab samples were tested for presence of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 using Digene Hybrid Capture 2 (hc2) according to the manufacturer’s instructions. Test positivity was defined as 1.0 RU/LCCO.

• We thank Digene Corp. for providing hc2 kits at reduced cost for this project.

• All hc2 positive samples, all women with a colposcopic exam, and a random sample of hc2-negative women without colposcopy were tested by PGMY09/11 consensus PCR and genotyped using the Roche prototype line blot (via kind donation from Roche Molecular Systems, Inc., Pleasanton, CA).


STATISTICAL METHODS

• Odds ratios and 95% confidence intervals were calculated using logistic regression

(State 9.8, College Station, TX)

Methods

Results

HPV 16-specific seroprevalence


• The lack of observed inflection point for both HPV seroprevalence and HPV DNA prevalence precludes an estimation of peak HPV exposure age.

• Indian culture does not encourage discussion of women’s sexual history. Assuming peak female exposure may occur in rural India at the time of marriage, we examined age at marriage and age at which women report first living with their husbands.

• Approximately 60% of women in our study reported being married and living with their husbands by age 15 years; 96% were married and living with their husbands by age 18 years.

• A substantial fraction of women reporting an earlier age of marriage relative to age first lived with their husband (see right graph). Further analysis of this cluster revealed a positive correlation in age at marriage and age first living with the husband in a subset of these women.

Determinants of HPV seroprevalence

• HPV 16 seroprevalence regardless of genotype. It is unknown whether this observation is explained by increased disease prevalence, evidence for equal distribution of ASCUS/L SIL across all ages tested, with slight increase in HSIL/CIN in women over age 45 years. Note, 58% of biopsy-confirmed CIN 2+ was detected in women under age 35.

Conclusions

• HPV 16 seroprevalence (19%) observed in rural India is similar to other population-based estimates in the US (18%, Stone K, et al JID 2002;186:1396-402) and Costa Rica (15%, Wang SS, et al Br J Cancer 2002;86:1248-1254), and lower than reported high risk populations such as STD clinics (30%, Thompson DL, et al JID 2002;190:1563-74) and HIV positive women (>50%, Viscidi RP, et al JID 2003;187:194-205).

• The peak age of exposure in India has not been demonstrated, and we were unable to provide additional information by seroprevalence estimates. The demographics of our population do suggest the possibility for a relatively young and narrow range age of exposure when using age at marriage as a surrogate for onset of sexual activity.

• Because of the homogeneity of the village populations, few unique determinants of HPV serostatus were identified. Active and passive tobacco exposure tended to be associated with an increased risk of HPV seroprevalence regardless of genotype. It is unknown whether this observation is explained by increased sexual exposures among women who use tobacco products or tobacco-associated immunosuppression.

• CIN 2+ prevalence in this population was low (1.3%). However, our data showing 58% of CIN 2+ in women under age 35 and the unknown peak age for HPV infection suggests that the age for once or twice in a lifetime screening in India might merit reconsideration.

• HPV 16 (19%) observed in rural India is similar to other population-based estimates in the US (18%, Stone K, et al JID 2002;186:1396-402) and Costa Rica (15%, Wang SS, et al Br J Cancer 2002;86:1248-1254), and lower than reported high risk populations such as STD clinics (30%, Thompson DL, et al JID 2002;190:1563-74) and HIV positive women (>50%, Viscidi RP, et al JID 2003;187:194-205).

• The peak age of exposure in India has not been demonstrated, and we were unable to provide additional information by seroprevalence estimates. The demographics of our population do suggest the possibility for a relatively young and narrow range age of exposure when using age at marriage as a surrogate for onset of sexual activity.

• Because of the homogeneity of the village populations, few unique determinants of HPV serostatus were identified. Active and passive tobacco exposure tended to be associated with an increased risk of HPV seroprevalence regardless of genotype. It is unknown whether this observation is explained by increased sexual exposures among women who use tobacco products or tobacco-associated immunosuppression.

• CIN 2+ prevalence in this population was low (1.3%). However, our data showing 58% of CIN 2+ in Women under age 35 and the unknown peak age for HPV infection suggests that the age for once or twice in a lifetime screening in India might merit reconsideration.

Funding support: International Agency for Research on Cancer (IARC, Lyon), an Indo-US collaborative grant from the Department of Biotechnology, Ministry of Science and Technology, Government of India and the NIH, USA

B71/IN/USCR/NPP/2002, and an NIH SPORE grant (PS0 CA82932)