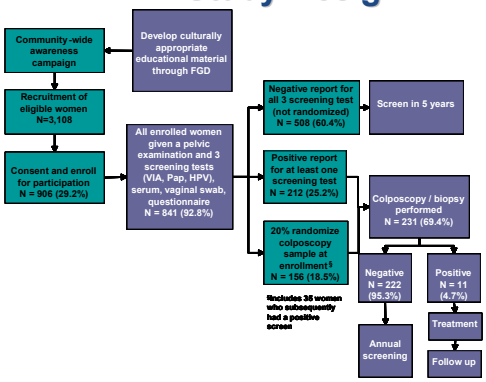
HPV DNA detection by PCR in clinician-collected cervical swabs and self-collected vaginal swabs in rural India

Sowjanya P[1], Paul P[2], Ramkrishna G[1], Bahwani K[3], Kumar P[1], Jain M[3], Das M[3], Reddy P[3], Vijayaraghavan K[3], Shah KV[2], Gravitt P[2*].
[1]Center for DNA Fingerprinting and Diagnostics, [2]Johns Hopkins Bloomberg School of Public Health, [3]MediCiti Institute of Medical Science, SHARE India

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 a the standard cervical sample, women were asked to provide a self-collected vaginal swab to evaluate the use of HPV-DNA testing on self-collected vaginal swabs as an alternative to clinic based screening

CATCH Study Design



Methods

Study design

This study is an interim analysis from a currently on-going 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
- intact uterus
- 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 (hc2)
- 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 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 RLU/CO.
  - *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).*
  - *Gravitt PE, et al. J Clin Microbiol 2000; 38:357-61*
  - *Gravitt PE, et al. J Clin Microbiol 1998; 36:3020-7*

Statistical Methods

Overall, agreement of high risk and type specific HPV results was estimated using kappa statistics. The Bayes formula was used for corrected sensitivity and specificity estimates for Pap, hc2, and VIA screening. The non-random sampling approach for PCR-based testing precluded our ability to generate corrected estimates.

Results

Demographics of population

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

	Total population (%)	Sample (%)
Overall	841	444
Age		
25-29	248 (29.5)	120 (27.0)
30-34	188 (22.3)	114 (25.7)
35-39	151 (18.0)	72 (16.2)
40-44	88 (10.5)	43 (9.7)
45-49	65 (7.7)	36 (8.1)
50+	101 (12.0)	59 (13.3)
Educated, self reported		
No	555 (66.0)	301 (67.8)
Yes	285 (33.9)	158 (32.0)
Age at marriage (in years)		
Don't Know	49 (5.8)	24 (5.4)
≤ 13	193 (22.9)	103 (23.2)
14-16	331 (39.4)	185 (41.7)
17+	268 (31.9)	132 (29.7)
Age at 1st pregnancy		
Don't Know	95 (11.3)	45 (10.1)
≤ 15	231 (27.5)	124 (37.9)
16-20	419 (49.8)	220 (49.6)
21+	96 (11.3)	55 (12.4)

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

Agreement of HPV DNA detection

Table 2: Agreement in HR-HPV DNA detection: Hybrid-capture 2 (hc2) *vs.* consensus PCR in cervical samples

	Consensus PCR†		
Hybrid Capture	HPV+	HPV-	Total
HPV+	76	12	88
HPV-	12	335	347
Total	88	347	435*

** 9 samples excluded because samples were β-globin negative.*
† Consensus PCR positive for 1 or more of the13 genotypes in the hc2probe set (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68).

- We confirmed the hc2 test results with L1 consensus PCR, confirming the validity of the local hc2 testing in India.
- The overall agreement is 94.4% (κ = 0.8, McNemar's p>1.0).
- Four HPV 16, 3 HPV 39, and one each of HPV 18, 52, 33, 59 and 31 were 'missed' by hc2.
- Of the hc2+/PCR negative samples, 3 were PCR positive with low risk types known to cross-react with hc2+ high risk probe pool (e.g., HPV 53 or 66), and 8 had an RLU/CO < 5.0 reflecting low viral load.

HPV Genotype Distribution

- In the general screening population, HPV 16 was the most commonly reported genotype, followed by HPV 52, 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

Table 3: Agreement in HR-HPV DNA detection between PCR-based HPV detection from vaginal and hc2 or PCR based HPV detection from cervical samples

	Cervical samples			
Vaginal samples *	hc2+	hc2-	PCR+	PCR-
HPV+	58	11	61	27
HPV-	29	335	8	335
Total†	87	346	69	362

** 11 vaginal samples excluded in hc2 based comparison (N=433) because the samples were β-globin negative. An additional 2 cervical excluded in PCR-based comparison (N=431) because 2 cervical samples were β-globin negative.*

- Vaginal swabs tested by L1 consensus PCR were highly concordant with both hc2 and PCR-based detection of HR-HPV from samples collected at the cervix.
- The concordance of vaginal swab PCR and hc2 was 90.8% (κ = 0.7, McNemar's p<0.01).
- The concordance of vaginal and cervical PCR-based HPV detection was 91.9% (κ = 0.7, McNemar's p<0.01).
- We observed 70.5% complete and 29.5% partial type-specific agreement between cervical and vaginal specimens, with no complete type discordance among high risk HPV types.

Sensitivity and Specificity Estimates

	Sensitivity		Specificity	
	Uncorrected	Corrected	Uncorrected	Corrected
Pap	62.5	50.0	79.3	86.4
VIA	22.2	9.4	82.9	93.9
hc2	77.8	61.9	81.5	90.5
HR-PCR , cervical	88.9	*	83.0	*
HR-PCR, vaginal	77.8	*	82.9	*

- 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 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-vaginal 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 sample 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 offer a practical alternative to cervical cancer screening in rural India.

We would like to acknowledge funding support from the 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 (BT/IN/US/CRHR/PP/2002), and an NIH SPORE grant (P50 CA98252). We would also like to thank Digene Diagnostics for competitive pricing of hc2 kits, and Roche Molecular Systems for the donation of PCR reagents.

Feasibility of field-based self sampling of adult women in Andhra Pradesh: Pilot study results from the CATCH Study

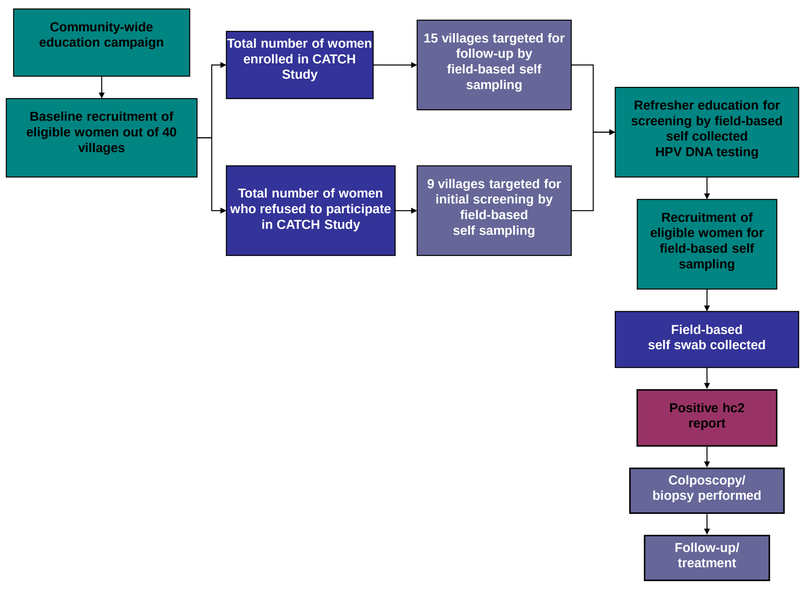
Paul P[1], Sowjanya P[2], Ramakrishna G[2], Haripriya V[3], Bhawani K[3], Das M[3], Reddy P[3], Vijayaraghavan K[3], Shah KV[1], Gravitt PE[1]

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

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.
- 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 93% (κ=0.8)
  - 70.5% complete and 29.5% partial type-specific agreement between cervical and vaginal specimens
- The objective of this study is to determine the feasibility of using field-based self collected vaginal sampling in the CATCH Study

Study Design



Methods

CATCH Study design

- CATCH Study eligibility –
  - 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=396; *median age = 36 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 went to a private area of their home 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

HPV DNA Detection

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 68 using Digene Hybrid Capture 2 (hc2) according to the manufacturer's instructions. Test positivity was defined as 1.0 RLU/CO.

Statistical Methods

Pearson's chi-squared and z-tests were used to determine difference in participation rates

Results

Population characteristics

Table 1. Characteristics of women who participated in cervical cancer screening by either initial clinic-based screening (baseline screened) or field-based self collected HPV DNA testing at second recruitment visit (newly screened) and total screened by either strategy (total screened)

	Baseline screened* (N=959)	Newly screened* (N=388)	Total screened* (N=784)
	Total N (% Enrolled)		
Age			
25-29	197 (54.3)	69 (65.2)	161 (73.9)
30-34	173 (55.5)	55 (63.6)	135 (79.3)
35-39	148 (53.4)	54 (63.0)	125 (77.6)
40-44	116 (48.3)	45 (46.7)	96 (66.7)
45-49	97 (38.0)	38 (18.4)	81 (54.3)
50+	228 (29.8)	127 (22.0)	186 (32.8)
Marital status			
Married	769 (47.0)	290 (50.7)	633 (80.7)
Divorced/Widowed	190 (32.1)	98 (23.5)	151 (19.3)
Family size (quartiles)**			
≤2	312 (33.0)	151 (25.8)	242 (40.9)
3	181 (42.0)	80 (40.0)	147 (68.7)
4	235 (59.2)	82 (54.9)	204 (72.6)
≥5	226 (58.4)	74 (62.2)	189 (75.7)
Education, self reported**			
None	772 (44.6)	349 (42.4)	651 (59.1)
Any	145 (61.4)	38 (57.9)	120 (81.7)
Occupation**			
Housewife	514 (41.6)	247 (40.5)	432 (58.3)
Laborer	308 (50.7)	117 (49.6)	260 (65.0)
Other	91 (68.1)	21 (52.4)	76 (79.0)
Village type			
Rural	521 (50.5)	209 (48.8)	438 (65.5)
Semi-rural	206 (38.8)	107 (35.5)	176 (56.8)
Semi-urban	232 (47.4)	72 (41.7)	170 (61.6)
Distance from hospital (km)			
0-8	375 (43.2)	175 (41.1)	318 (62.3)
9-16	499 (49.5)	174 (47.7)	391 (64.5)
17+	85 (51.8)	39 (38.5)	75 (56.0)

Baseline population*: total number of women from 15 villages approached for initial enrollment (clinic-based) screening. ***Newly screened population*: total number of women who refused initial clinic-based screening with the opportunity for field-based self HPV collection. ***Total screening population***: total number of women with the opportunity of both initial enrollment (clinic-based) screening and a field -based self HPV collection*

***Numbers don't add up to total due to missing values*

Focus Group Discussion

Table 2. Summary of FGD: Acceptance of field based self collected HPV DNA testing program

General attitudes toward field based self sampling

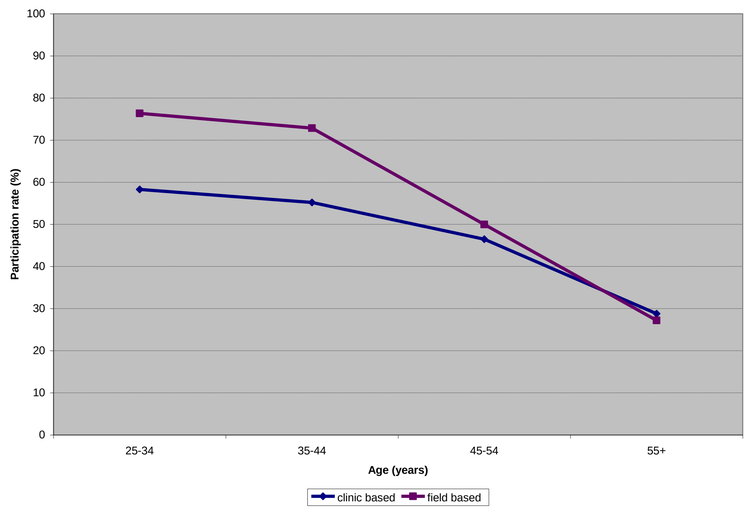
- Majority accepted the idea of field based testing
  - *"Coming to village for testing is good"*
  - *"Going to the hospital…we will be loosing one day wage"*
- Reaction towards self sampling
  - Initially felt *"shy"* and *"funny"*, but still will to perform the test
  - Comfortable performing the test after someone explains the procedures
  - Still feels doctor will perform the test better.

Logistical concerns for field-based self sampling

- Majority preferred to participate in screening early morning or evenings
  - *"I would like to collect swabs immediately before 8am…..or in the evening after returning from the fields…"*
- Some expressed concern about appropriate location for sample collection
  - *"No, we won't feel comfortable using the self-swab at home because we don't have proper bathroom facilities*
  - *"…we don't have any PHC [primary health centers]…"*
- When asked about report distribution, no consensus was reached.
  - Some women would rather collect reports from the clinic since they may have an opportunity to meet with the doctor, find out what is wrong and get *"medicine."*
  - Others would like the reports distributed in the field and only go to the hospital is there is a problem.
  - Women want to discuss their health problems with a doctor, but willing to talk with *"counselors or sisters [nurses/Health supervisors]…"* because doctors *"will always be busy…"*

Participation Rates

Figure1. Age-specific participation in clinic-based screening and field-based self collected HPV swab



- 63% provided an at-home self collected sample (*median age = 35 years*)
- The initial participation rate (50%) for the clinic-based screening study was significantly lower (p<0.01)
- Among women targeted for once of twice in a lifetime screening (35-45 years), 85% were screened once either by clinician collected or self collected HPV testing

Preliminary HPV natural history

Table 3. Follow-up of detection of HPV by hc2: incidence, persistence, and clearance rate

	% (95% CI)
Incidence	3.8% (2.0, 6.5)
Persistence	19.0% (5.4, 41.9)
Clearance	81.0% (58.1, 94.6)

- Out of 21 hc2 positive women at baseline:
  - 17 women (81.0%) cleared any HPV infection
  - 4 women (19.0%) have persistent HPV infection
- Out of the 317 hc2 negative women at baseline:
  - 12 (3.7%) have a new HPV infection

Conclusions

- Field-based self sampling is a feasible alternative to improve population coverage for cervical cancer screening in rural India
- Using once or twice in a lifetime screening strategy targeting women aged 35 or 35 & 40 years coverage via home-based self sampling for HPV detection is estimated at approximately 85% relative to 60% if screening required clinic-based speculum examination
- Good compliance with self-swab among previous CATCH participants at follow-up (81.3%) suggests feasible means to study HPV natural history
- A notable percentage of women (19%) had a persistent HPV infection at follow-up, identifying a target population for twice in a lifetime screening strategy.
- Rates of incidence, persistence and clearance were calculated from cervical-vaginal paired hybrid capture results and therefore may not indicate true estimates, however the concordance of PCR detection between physician-collected cervical swabs and self-collected vaginal swabs is high. To verify, we will test paired clinic-based and field-based self collected vaginal samples by PCR-based HPV detection.
- Further cervical cancer screening programmatic research will focus on follow-up methods for informing and referring women with positive HPV test results for increased compliance with colposcopy and treatment
- Use of newly developed rapid HPV tests in a field-based screen and treat scenario might offer an alternative strategy for cervical cancer screening

We would like to acknowledge funding support from the 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 (BT/IN/US/CRHR/PP/2002), and an NIH SPORE grant (P50 CA98252). We would also like to thank Digene Diagnostics for competitive pricing of hc2 kits, and Roche Molecular Systems for the donation of PCR reagents.

Community Access to Cervical Health – the CATCH Study
Results from a pilot screening efficacy study.

Jain M[1], Paul P[2], Hariharan S[1], Ratnakar C[1], Kumar P[1], Reddy P[1], Nath N[1], Das M[1], Sowjanya P[3], Ramakrishna G[3], Bhatla N[4], Rosenthal D[2], Shah KV[2], Gravitt P[2*].
[1]MediCiti Hospitals, [2]Johns Hopkins University, [3]Center for DNA Fingerprinting and Diagnostics, [4]All India Institute of Medical Sciences

Background

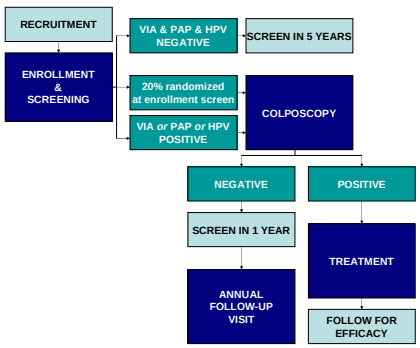
- India bears one-fifth of the global cervical cancer disease burden.
- Evidence of effective screening programs is seen in developed countries to reduce the burden and impact of cervical cancer
- Negative perceptions about visiting the doctor when asymptomatic are barriers to screening programs in India.
- Compliance with[elvic examination is a predominate 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

Recruitment

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

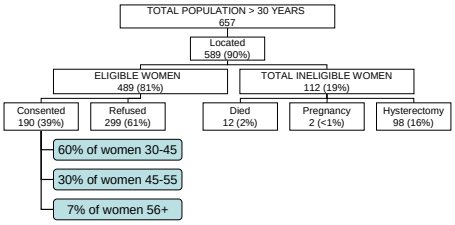
The following was collected at enrollment:
- Written informed consent
- Questionnaire (demographics, reproductive history, contraceptive history, tobacco use, medical history)
- Blood (10 mL serum)
- Self-collected vaginal swab (Digene Sampler Kit)
- Pelvic exam
  - Pap smear (Ayre's spatula and endocervical brush fixed in 95% EtOH)
  - Clinician-collected cervical swab (Digene Sampler Kit)
    - HPV testing by Hybrid Capture 2, high risk probe pool
  - VIA performed by gynecologist (training at Barshi Cancer Cervix Prevention Project, an IARC study site).
- 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
- Biopsy only suspicious lesions; histopathology results hand-delivered and CIN2+ scheduled for treatment.

Results

Participation



Reasons for non-participation

Reason for refusal	N	%
No reason reported	164	(54%)
Screening not important	28	(20%)
No time, inconvenient	21	(15%)
Fear a cancer diagnosis	16	(12%)
Confidentiality concerns*	14	(10%)
Distrust of doctors	3	(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 from these discussions highlighted several issues that significantly affect willingness of rural Indian women to participate in cervical cancer screening programs.

1. Reluctance to visit the doctor if asymptomatic

This was a pervasive concern in focus group discussions and from anecdotal observations of field staff. Women believe that this exam is unnecessary and fear that the doctors are 'removing their womb for no reason'. Most women reported that they would not seek gynecologic care in the absence of symptoms, even for ante-natal care.

2. Conceptual understanding of pre-invasive disease

Women seemed unable to understand the concept of having a 'pre-cancer'. They do not believe that cervical cancer can be prevented, and a positive screening test is frequently misinterpreted as a cancer diagnosis. Even in the latter case, women often felt resigned to the cancer as their fate, and refused treatment of the neoplasia.

3. Husbands refusal

Many women cite disapproval by husbands as a reason for not participating in screening or follow-up.

4. Cost not an issue

Despite provision of free services, transportation, and referral for other medical needs, women were reluctant to participate. When probed specifically, cost was not a significant issue in the choice to participate among focus group participants.

5. Previous lack of consideration by hospital staff

Bad experiences at the local hospital in the past created a reluctance to participate in the cervical cancer screening project.

Demographics of enrolled population

	N	%
Age		
30-35	48	25.4
36-40	49	25.9
41-45	36	19.1
46+	56	29.6
Ever attend school?		
No	134	70.5
Yes	56	29.5
Inside toilet?		
No	40	26.3
Yes	150	73.7
Marital status		
Married	170	89.5
Divorced	2	1.1
Separated	5	2.6
Widowed	13	6.8
Age at marriage (years)¥		
≤ 13	47	24.7
14-16	101	53.2
17+	42	22.1
Menopausal status		
premenopausal	136	72.7
postmenopausal	51	27.3
Age at 1st pregnancy (years)*		
≤ 15	52	27.4
16-20	110	57.9
21+	19	9.0
Parity		
0-2	55	29.0
3-4	90	54.7
5+	36	11.6
Ever used birth control?		
No	36	19.0
Yes	154	81.0
Tubal ligation		
No	4	2.6
Yes	150	97.4
Age at tubal ligation (years)		
≤ 20	17	11.3
21-25	64	42.4
26+	50	33.0
can't remember	20	13.3
Ever had a Pap smear?		
No	148	79.6
Yes	8	4.6
Don't know	30	16.1

Table 3. Age-stratified screening prevalence

AGE	N	VIA	PAP	HPV
30-35	45	6 (13.3)	4 (8.9)	6 (13.3)
36-40	46	2 (4.4)	7 (15.2)	4 (8.7)
41-45	36	2 (5.6)	6 (16.7)	2 (5.56)
46+	51	0	24 (47.1)	6 (11.8)

•Only 1 patient was positive by all 3 screening tests
•Based on these screening results – 62 women (32.6%) were referred to colposcopy, of whom 35 (56%) returned.
•Of these women, 3 were confirmed as having CIN 1 and 3 were confirmed as having CIN 2 lesions on biopsy

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 in 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 post 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 INDO-US collaborative grant from the Department of Biotechnology, Ministry of Science and Technology, Government of India and the NIH, USA (BT/IN/US/CRHR/PP/2002), and an NIH SPORE grant (P50 CA98252). We would also like to thank Digene Diagnostics for competitive pricing of hc2 kits, and Roche Molecular Systems for the donation of PCR reagents.

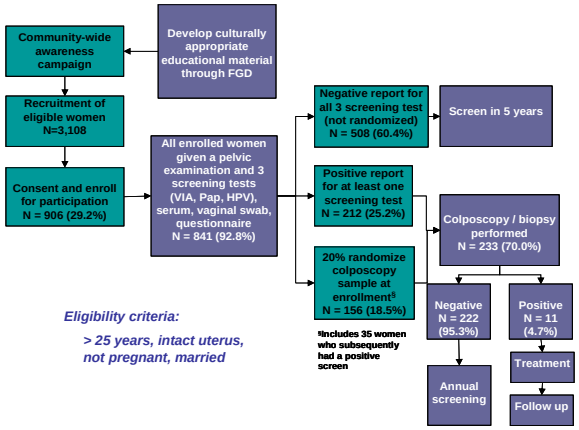
Seroepidemiology of HPV 11, 16, and 51 in a population-based sample of adult women in rural India

Gravitt P[1*], Paul P[1], Sowjanya P[2], Ramkrishna G[2], Ratnakar N[3], Bahwani K[3], Jain M[3], Das M[3], Reddy P[3], Vijayaraghavan K[3], Shah KV[2], Viscidi R[1].
[1]Johns Hopkins University, [2]Center for DNA Fingerprinting and Diagnostics, [3]MediCiti 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



Eligibility criteria:
> 25 years, intact uterus, not pregnant, married

Methods

HPV SEROLOGY

- HPV 16 serostatus was determined by use of an HPV 16 Virus-Like Particle (VLP) ELISA.
  - *Viscidi R, et al. J Infect Dis 2003; 187:194-205.*
- Cut points for seropositivity were defined as OD values five standard deviations above the mean value obtained from negative control sera referenced to seroreactivity of children.

HPV DNA DETECTION

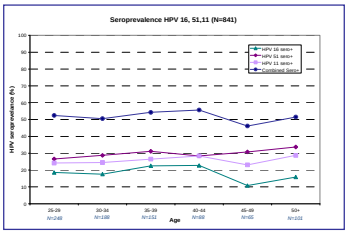
- All cervical swab samples were tested for presence of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 using Digene Hybrid Capture 2 (hc2) according to the manufacturer's instructions. Test positivity was defined as 1.0 RLU/CO.
  - *We thank Digene Corp. for providing hc2 kits at reduced 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).*
  - *Gravitt PE, et al. J Clin Microbiol 2000; 38:357-61*
  - *Gravitt PE, et al. J Clin Microbiol 1998; 36:3020-7*

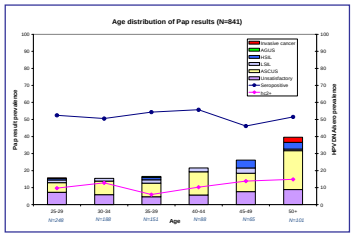
STATISTICAL METHODS

- Odds ratios and 95% confidence intervals were calculated using logistic regression (Stata 9.0, College Station, TX)

Results

Markers of HPV exposure by age

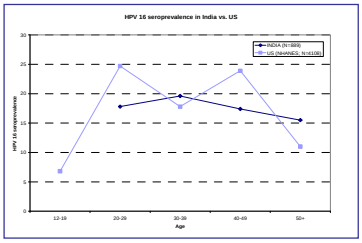


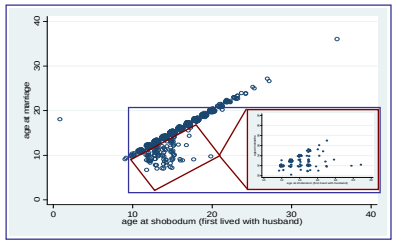


- Average Seroprevalence: HPV 11=25.6%, HPV 16=18.6%, HPV 51= 29.3%
- 51.1% of women were seropositive to 1 or more of the HPV types tested (combined seropositive).
- Seroprevalence did not vary substantially by age, except for the slight decline in HPV 16 seroprevalence around age 45 years).
- High-risk (HR) HPV DNA average prevalence of 10.7%.
- Using cytologic diagnosis as surrogate for disease prevalence, evidence for equal distribution of ASCUS/LSIL across all ages tested, with slight increase in HSIL/ICC in women over age 45 years. *Note, 58% of biopsy confirmed CIN 2+ was detected in women under age 35.*

Results, cont.

HPV 16-specific seroprevalence





- Age-specific HPV 16 seroprevalence in rural India (2005) is similar to a national US survey conducted 1988-1994 *(Stone K, et al JID 2002;186:1396-402*).
- 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; 86% 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 menarche and age first living with the husband in a subset of these women.

Determinants of HPV seroprevalence

	N	HPV 11 OR (95% CI)	HPV 16 OR (95% CI)	HPV 51 OR (95% CI)	Any HPV OR (95% CI)
Age (years)					
25-30	248	ref	ref	ref	ref
30-34	188	0.8 (0.5 - 1.4)	0.8 (0.4 - 1.4)	0.9 (0.5 - 1.6)	0.9 (0.5 - 1.4)
35-39	151	0.8 (0.5 - 1.4)	0.7 (0.4 - 1.4)	1.0 (0.6 - 1.8)	0.8 (0.5 - 1.4)
40-44	88	0.9 (0.5 - 1.6)	1.0 (0.5 - 1.9)	1.1 (0.6 - 2.0)	0.9 (0.6 - 1.6)
45-59	65	0.8 (0.4 - 1.6)	**0.4 (0.2 - 1.0)**	1.1 (0.6 - 2.3)	0.7 (0.4 - 1.3)
50+	101	1.0 (0.5 - 1.9)	0.6 (0.3 - 1.3)	1.3 (0.7 - 2.4)	0.8 (0.5 - 1.5)
Occupation					
housewife	231	ref	ref	ref	ref
agriculture	219	0.8 (0.5 - 1.2)	1.6 (0.9 - 2.5)	0.9 (0.6 - 1.4)	0.9 (0.6 - 1.4)
labor	61	0.7 (0.3 - 1.3)	1.6 (0.8 - 3.3)	1.7 (0.9 - 3.0)	1.0 (0.6 - 1.8)
private job	49	0.6 (0.3 - 1.4)	1.5 (0.7 - 3.4)	1.3 (0.7 - 2.4)	1.2 (0.6 - 2.2)
other	281	0.9 (0.6 - 1.3)	1.5 (0.9 - 2.3)	0.9 (0.6 - 1.3)	0.9 (0.6 - 1.3)
Have an inside toilet?					
No	285	ref	ref	ref	ref
Yes	554	1.3 (0.9 - 1.8)	0.9 (0.6 - 1.3)	**0.8 (0.6 - 1.0)**	1.1 (0.8 - 1.5)
Marital Status					
married	739	ref	ref	ref	ref
divorced/separated/widowed	98	0.9 (0.5 - 1.4)	1.1 (0.7 - 1.9)	**1.6 (1.0 - 2.4)**	1.0 (0.7 - 1.5)
Age first lived with husband (years)					
<14	193	ref	ref	ref	ref
14-16	331	0.8 (0.5 - 1.2)	0.8 (0.5 - 1.3)	**1.5 (1.0 - 2.3)**	1.0 (0.7 - 1.4)
>16	268	0.9 (0.6 - 1.4)	0.9 (0.6 - 1.5)	1.1 (0.7 - 1.7)	1.0 (0.7 - 1.5)
Age at menarche (years)					
< 12	82	ref	ref	ref	ref
12-13	570	0.8 (0.5 - 1.3)	1.4 (0.7 - 2.8)	0.8 (0.5 - 1.4)	1.0 (0.7 - 1.6)
>13	166	0.9 (0.5 - 1.6)	1.8 (0.9 - 3.7)	0.7 (0.4 - 1.3)	1.0 (0.6 - 1.7)
Parity					
0-2	351	ref	ref	ref	ref
3-4	384	1.0 (0.7 - 1.3)	1.1 (0.8 - 1.6)	**1.4 (1.0 - 2.0)**	1.3 (0.9 - 1.7)
5+	106	0.9 (0.5 - 1.4)	1.3 (0.8 - 2.3)	1.2 (0.7 - 1.9)	1.0 (0.6 - 1.5)
Tobacco Use					
never	734	ref	ref	ref	ref
ever	107	**1.6 (1.0 - 2.4)**	1.2 (0.8 - 2.0)	**1.5 (1.0 - 2.3)**	1.4 (0.9 - 2.2)
Live with a smoker?					
No	475	ref	ref	ref	ref
Yes	366	**1.6 (1.1 - 2.1)**	0.9 (0.6 - 1.3)	1.2 (0.9 - 1.7)	**1.5 (1.1 - 1.9)**

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 2003;89:1248-1254*), 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 age range 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 (BT/IN/US/CRHR/PP/2002), and an NIH SPORE grant (P50 CA98252).