Performance of HPVDNA Test in presence of co-infection with common reproductive tract infections

By
Dr. KAVITA VIVEK ANAND

HLTH09201304007

Tata Memorial Centre
Mumbai

A thesis submitted to the

Board of Studies in Health Sciences

In partial fulfillment of requirements

for the Degree of

DOCTOR OF PHILOSOPHY

of

HOMI BHABHA NATIONAL INSTITUTE

July 2020

Homi Bhabha National Institute[1]

Recommendations of the Viva Voce Committee

As members of the Viva Voce Committee, we certify that we have read the dissertation prepared by Dr. Kavita V. Anand entitled '**Performance of HPVDNA Test in presence of co-infections with common reproductive tract infections**' and recommend that it may be accepted as fulfilling the thesis requirement for the award of Degree of Doctor of Philosophy.

Chairperson -Dr. Amita Maheshwari — Signature with date 7/7/2020

Guide- Dr. Sharmila A. Pimple — Signature with date 29/06/2020

Examiner- Dr. Ranajit Mandal — Signature with date 07/8/2020

Member 1- Dr. Shyam K. Shrivastava — Signature with date 25/06/2020

Member 2- Dr. Surendranath S. Shastri — Signature with date June 24, 2020

Member 3- Dr. Gauravi A. Mishra — Signature with date 30/6/20

Technical Adviser – Dr. Preeti R. Mehta — Signature with date PRMehta 1.7.20

Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to HBNI.

We hereby certify that we have read this thesis prepared under our direction and recommend that it may be accepted as fulfilling the thesis requirement.

07/07/2020

Guide: Signature &Date

[1] This page is to be included only for final submission after successful completion of viva voce.

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at Homi Bhabha National Institute (HBNI) and is deposited in the Library to be made available to borrowers under rules of the HBNI.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the Competent Authority of HBNI when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

KAnaidu

Dr Kavita.V. Anand

15/6/2020

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at Homi Bhabha National Institute (HBNI) and is deposited in the Library to be made available to borrowers under rules of the HBNI.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the Competent Authority of HBNI when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

KAnand

Dr Kavita.V. Anand

15/6/2020

CERTIFICATE

I certify that the thesis titled 'Performance of HPVDNA Test in presence of co-infections with common reproductive tract infections' submitted for the degree of Doctor of Philosophy by Kavita.V.Anand is record of original research work carried out by her under my supervision. This work has not formed the basis for the award of any degree, diploma, associateship or fellowship at this or any other institute or university.

07/07/2020

Dr Sharmila. A. Pimple
Guide, Professor &Physician,
Department of Preventive Oncology,
Tata Memorial Hospital,
Parel, Mumbai- 400012.

LIST OF PUBLICATIONS & ACADEMICS

Publication as a part of thesis

1. Anand KV, Pimple SA, Mishra GA, Sahare RV, Pathuthara S, Deodhar KK, Shastri SS. Reliability of conventional Papanicolaou smear in diagnosing bacterial vaginosis among women with clinical genital infection. South Asian J Cancer. 2020;9:13-6.

Other publications

1. Anand KV, Mishra GA, Pimple SA, Deodhar KK, Kelkar RS, Dighe S et al. Comparative Evaluation of Results of Pap smears and HPV Hybrid Capture 2 Performed on cervical sample before and after application of acetic acid. International Journal of Health Sciences & Research. 2019;9:34.

2. Anand KV, Mishra GA, Pimple SA, Pathuthara S, Kulkarni V. 'Detection of Rare Parasite on Pap Smear'. Accepted Cytojournal.

Paper presented

1**.** Presented on 'Women's Cancer Prevention, Screening and Early Detection in '17th Joint State Conference of IAPSM & IPHA, MHIAPSMIPHACON-2016 in association with Mumbai branch of IAPSM- Feb 2016 at Tata Memorial Hospital.

2. Scientific paper presented on 'Pilot study to investigate the effect of Acetic acid on Pap smear and HPV Hybrid Capture 2 test in cervical cancer screening' at Kolkata Health Summit – Sep 2017 held at Kolkata, India.

Other Conferences attended as delegate /faculty / trainings underwent during the academic years attached as Annexure-2

.

ACKNOWLEDGEMENTS

I take this opportunity to thank the almighty to have blessed me with excellent mentors, Dr Surendra Shastri, Dr Sharmila Pimple, Dr Gauravi Mishra. My special gratitude to Dr Surendra Shastri for giving me an opportunity to accomplish Ph.D. He has steered me past every obstacle during the course. I consider him as a figure of true mentor. My deep gratitude to have Dr Sharmila Pimple as my guide, who has mentored me at every step, stood firmly besides me in all difficult times and as a person taught me the importance of spirituality in daily living. Blessed to have Dr Gauravi Mishra, who I always looked upon as my senior, always encouraged and helped me throughout the journey of my Ph.D. course. I thank them all for bringing out the best in me.

I shall never forget the role of my parents who were my true pillars of support in this journey. I thank my parents for being there for me through all good and bad stages of my life. There are no boundaries to express my gratitude towards them, for they are my world.

I thank my Doctorate Committee members, Dr. Shyam Shrivastava, Dr. Amita Maheshwari and Dr. Preeti Mehta for guiding and supporting me to learn the principles of conducting a good clinical research. I consider myself to be fortunate, as very few people are blessed with this opportunity of learning from expertise in their field. I would like to extend my special thanks to Dr Atanu Bhattacharjee for carrying out statistical analysis of my study.

I thank director Dr.R. Dikshit for lending me the opportunity to be a student of the most prestigious Institute. My sincere thanks to Dean Academics, Dr. S. Banawali for the support given to accomplish my curriculum. I express my sincere thanks to Ms. Kashikar from Academic Department, Ms. Deepali Parabh & Mr. Laurence D'Souza from Department of Preventive Oncology for helping me with official work of academics which is one of the most crucial tasks in any Ph.D. programme.

I thank my colleagues Dr. Pramod Kharade, Dr. Vasundhara Kulkarni, Dr. Heena Sheikh and Dr. Saurabh Bobdey to extend their strong support in helping me complete my Ph.D. I thank the entire staff of the Preventive Oncology Department to help me in every way to make my 7-year journey a success. Special thanks to Ms Darshana Rane, Ms Rupa Bhavsar, Ms. Sheela Sardara, Ms. Tina Solanki for helping me in managing the microbiological part of the study.

Last but not the least, I dedicate my work to my daughter Rutika Anand and husband Vivek Anand who are my treasures of life for caring and being pillars of strength throughout this tenure. Without their support and love, I would have never acquired the most prestigious professional degree of my life. I extend my sincere thanks to my friend Ms. Ruknaz Wadia for taking care of my daughter in times of crisis.

MILESTONES OF THE STUDY

Study presented before 1st Doctorate Committee meet	December 15th 2015
Study presented in DMG Gynaec-Onco, Tata Memorial Hospital	December 16th 2015
Study received Institutional Review Board clearance from Tata Memorial Hospital	June 14th 2016
Study submitted to Institutional Ethics Committee, H. B. T. Medical College & Dr. R. N. Cooper Hospital for approval	June 30th 2016
Study registered with Clinical Trial. gov NCT02830230	July 8th 2016
Study presented before Institutional Ethics Committee H. B. T. Medical College & Dr. R.N. Cooper Hospital	July 15th 2016
Study received Institutional Ethics Committee clearance from H.B.T Medical College & Dr. R.N. Cooper Hospital	July 15th 2016
Study qualified for TMC Research Administration Council Funding	July 25th 2016
Start date of Study at Tata Memorial Hospital	August 8th 2016
Start date of study at H. B. T. Medical College & Dr. R. N. Cooper Hospital	October 24th 2016
Study presented before 2nd Doctorate Committee meet	December 15th 2016
Study presented before 3rd Doctorate Committee meet	December12th 2017
Pre synopsis Doctorate Committee meet	April 23th 2019.

Abstract

Performance of HPVDNA Test in presence of co-infection with common reproductive tract infections.

Background- Molecular Hybrid Capture 2 test (HC2) is accepted screening modality for cervical cancer in developed countries as the test demonstrates a good sensitivity (average 95%). Presently, the issue with the HPVDNA HC2 test in implementing as a primary screening test in developing countries is its low sensitivity reported (Average 79%). The multicentric study from India reported the HC2 sensitivity of 68.2% which was substantially lower as compared to developed countries. India has a huge burden of untreated reproductive tract infections (RTIs).

Objective- To study whether the presence of clinical cervicitis or co-infections with Lab diagnosed RTIs interfere with the test result of HPV DNA by HC2 method.

Study design- Case-control study

Method- A total of 508 women, 254 women with clinical cervicitis and 254 asymptomatic women without cervicitis were enrolled as cases and control respectively. A baseline cervicovaginal swabs for RTIs and HC2 test samples were collected for all women enrolled in the study. All women in case arm received syndromic treatment for cervicitis while women in control arm received no treatment. A repeat cervicovaginal swabs and HC2 test was collected for all women enrolled in the study after 7-14 days.

Results & Conclusion - The present study demonstrated, the overall detection rates of HPV by HC2 test to improve by 4.5% among women treated for mucopurulent cervicitis. On controlling the biological and behaviour determinants by modelling, the study demonstrated influencing role of mucopurulent discharge associated with cervicitis on the test results of HC2 test.

INDEX

SYNOPSIS

Performance of `HPVDNA Test in presence of co-infection with common reproductive tract infections

Introduction

Cervical cancer has a precancerous stage that can be diagnosed by simple and cost-effective screening test. It still remains fourth common cancer among women globally and second common cancer among Indian women.[1,2] There is a strong causal association between persistence of cervical oncogenic Human Papilloma Virus (HPV) and cervical cancer.[3,4]

The robust study from India have shown that single round of HPV DNA screening test to significantly reduce the cervical cancer mortality.[5] The above findings are very encouraging since it demonstrates that a simple and reliable HPV DNA Hybrid Capture 2 test (HC2) which is now available in low income countries has a potential to be accepted as primary screening test in future. The cross-sectional studies from developed countries [6-14] from year 1999-2004 which focused on test characteristics of HC2 test to determine Cervical intraepithelial neoplasm grade 2 and above lesions (CIN2+), demonstrated test sensitivity in the range of 90% to 100% with an average of 95%.

Similarly, the cross-sectional studies from developing countries [15-21] from year 1993 to 2003 which focused on test characteristics of HC2 test to determine CIN2+ lesions, have shown a sensitivity in the range of 50% to 91% with an average of 79%. Among the developing countries cross-sectional study from India which evaluated test characteristics of HPV DNA HC2 in detecting CIN-2+ lesions showed a substantially low sensitivity of 68.2%.[20] The study from Mumbai which was a part of the multicentric study demonstrated a sensitivity of 62%.[22]

There is a statistically significant difference (p value = 0.003) of sensitivity of cervical HPV DNA HC2 test between the developed and developing countries to determine CIN2+ lesions while there is no difference in the specificity. Various reasons for low sensitivity from

developing countries have been discussed like fluctuations in temperature during transportation that can lead to denaturation of HPVDNA, improper sampling technique of collecting cervical HPVDNA, verification biases, prevalence of HPV in a particular geographical area and availability of HC2 probes of the common HPV types present in a particular area.[15-22]

The burden of RTIs are expected to be high among middle- and low-income countries as these countries lack development of quality care and treatment for STIs/RTIs (sexually transmitted disease/reproductive tract infections) services due to resource constrains as compared to high income countries. The Mid -term review of STI services by National AIDs control Program India (NACO) in 2009 reported 8.2 million episodes of STIs treated. The most common bacterial RTIs reported from India is Bacterial vaginosis followed by Candidiasis.[23] In middle income country like India, the burden of RTIs is expected to be higher than reported in the literature due to factors like illiteracy, ignorance, cultural norms, limited health care facilities for screening RTIs and poor access to health care facilities if available.[24,25]

Cervical cancer screening tests can have a potential to be affected by inflammation caused by RTIs. Liu W et al conducted a study among Chinese women to detect the prevalence of HPV genotype among three groups of women, group 1- mucopurulent cervicitis, group 2-healthy women and group 3- women with Invasive cancer. The author reported a 10% higher failure rates to extract HPV DNA in cases of mucopurulent cervicitis as compared to other two groups.[26]

Since the potential of any primary screening test to be adopted by a country depends on the sensitivity of the test, it becomes important to address the issue of sensitivity of HC2 test from Indian context. HPVDNA HC2 test is considered as a gold standard test to diagnose HPV infection, but the limitation of the test is, there is no inbuilt mechanism to monitor cell adequacy.[27] The current study explored, if cervicitis caused due to RTIs resulting in

mucopurulent discharge is hampering the detection rates of cervical HPV leading to false negative test results of HC2 test. This may be one of the factors affecting the sensitivity of the test due to cell inadequacy.

Aim of the study

To study if concomitant RTIs leading to cervicitis interfere with performance of HPVDNA HC2 testing

Objective of study

A] Primary objective

1. To study whether the presence of clinical cervicitis /or co-infections with Lab diagnosed RTIs interfere with the result of HPV DNA testing by HC 2 method.

B] Secondary Objective

1. To determine the prevalence of HPV infection in women with and without clinically diagnosed cervicitis.

2. To determine prevalence of HPV infection in women with lab diagnosed RTIs. (Gonococcal, Non- Gonococcal infections, Bacterial Vaginosis, Candida)

Ethical clearance

The study was a collaborative study between Department of Microbiology, Unit-1 of Obstetrics and Gynecology Department of H.B.T.M. College & Dr R. N. Cooper Hospital. (Cooper Hospital) with Preventive Oncology Department of Tata Memorial Hospital (TMH). The study protocol was reviewed and approved by the ethics committees and institutional review board of the participating centres respectively. The study was initiated after registering with Clinical Trial.gov. Clinical Trial.gov.NCT02830230

Study Design

Case- Control

Criteria for cases

Sexually active non-pregnant women aged 30–50 years with clinically diagnosed unhealthy cervix with signs of cervical inflammation that bleeds on touch associated with mucopurulent or purulent discharge (mucopurulent cervicitis) present on per speculum examination, irrespective of symptoms.

Criteria for controls

Sexually active, non-pregnant women aged 30–50 years, asymptomatic pertaining for RTIs complaints and no signs of cervicitis or vaginitis on per speculum examination.

Exclusion criteria for study

1.The women who received any antibiotics or treatment for reproductive tract infection (RTIs) within the last 4 weeks before enrolment.

2. Women with present or past history of treatment for cervical cancer.

3. Women with only vaginitis without cervicitis for the case arm.

4.Women with history of drug allergy to treating drugs (Cefixime, Azithromycin, Secnidazole, Metronidazole, Doxycycline, Fluconazole).

5.Women not willing to follow up

6.Women currently on antitubercular drugs, or with a known history of immunodeficiency syndrome.

7.Women who received any HPV vaccination in the past.

Sample Size –(calculated by nMaster 2.0 software)

The prevalence of cervical HPV in general population of India is estimated to be 7.1-10.3%.[28,29] In order to detect 10% difference in cervical HPV detection rates by HC2 test after

treatment of mucopurulent cervicitis, with 80% power and error of 5%, the sample size estimate was165 women. Further assuming an attrition rate of 20% for treatment failure and 15% loss to follow up the sample size was worked out to be 254 women. Thus, a total of 508 women, 254 with cervicitis (case arm) and 254 asymptomatic and without cervicitis/vaginitis (control arm) were enrolled into the study.

Study setting

In the present Study the women were enrolled from Unit-1 of Obstetrics and Gynecology Department of Cooper Hospital and Department of Preventive Oncology Tata Memorial Hospital (TMH). All the women enrolled at Cooper Hospital were registered with Preventive Oncology Department, TMH for further evaluation and processing of HPV samples. The cervicovaginal slides collected for lab diagnosis of RTIs were heat fixed and transported to Microbiology Department of Cooper Hospital for Gram staining and HPVDNA samples were sent to Microbiology Department of TMH for further processing. The microbiologists were blinded to the allocation of the women to case/control arm.

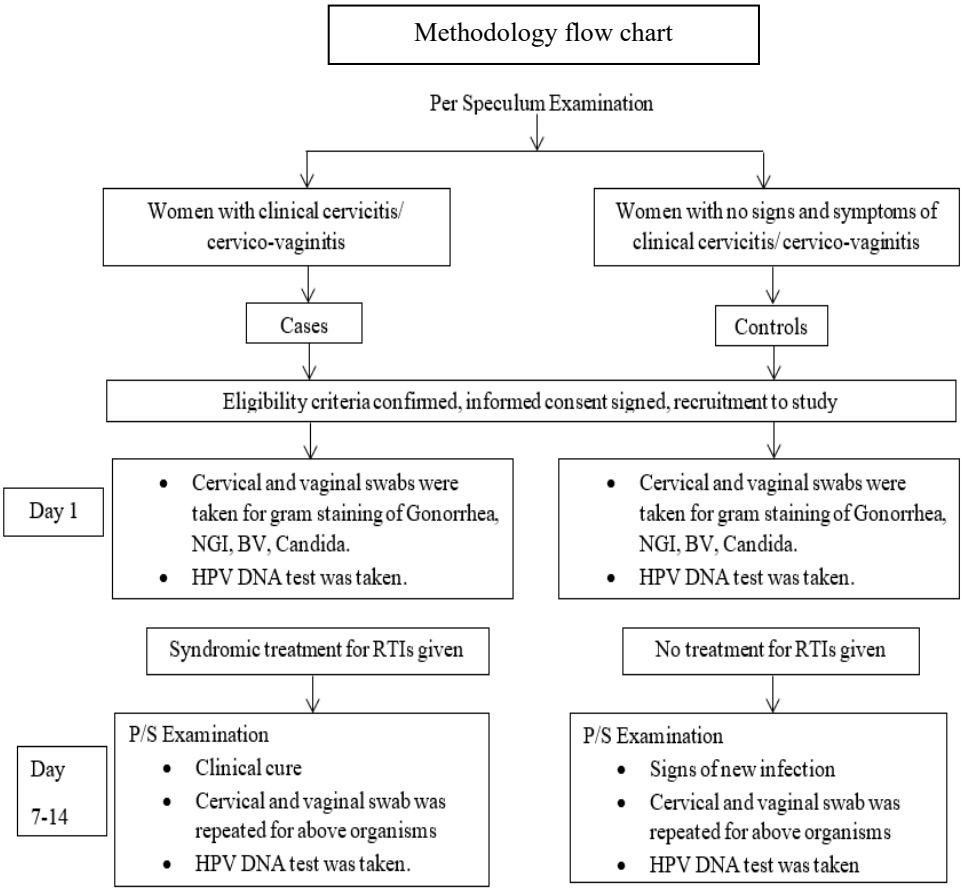
Material and methods

Among women visiting the Departments of Preventive Oncology (TMH) and Obst & Gynae (Cooper Hospital) during the period of August 2016 to August 2018, sexually active, non-pregnant women in the eligible age group of 30-50 years were counselled about the study and invited to participate in the study. The women who satisfied the eligibility criteria were consented for recruitment in the study. Among these, women with clinical mucopurulent cervicitis on per speculum examination (P/S) were enrolled under case arm and asymptomatic women without clinical cervicitis/vaginitis were enrolled under control arm. The women were then interviewed for sociodemographic data, reproductive history, medical history and symptoms pertaining to RTIs which was captured on a prestructured validated proforma. Smears of cervicovaginal discharge for Gram stain followed by HPVDNA HC2 test were

collected for all the women enrolled in the study. A per vaginum examination (P/V) was done to rule out pelvic inflammatory disease (PID). All the women enrolled in the case arm received syndromic treatment while the women in control arm received no treatment. All women enrolled in the study were asked to follow up between 7-14 days after the first visit and were counselled for sexual abstinence or use of barrier contraception (condoms) till she reports for her second follow up.

Instructions for follow up – Women in the case arm were advised to stop the vaginal pessary at least 3 days before she reports for the second sample collection and no samples were collected during menstrual cycle.

On follow up visit, the compliance for sexual abstinence/use of barrier contraception were noted for all women. A repeat P/S was done and clinical finding were noted. (for clinical cure of cervicitis among women in case arm/signs of new infection among women in the control arm.) A repeat cervico-vaginal swab followed by HPVDNA HC2 test were collected for all the women. A repeat P/V was done to assess the clinical cure among the women with PID at baseline visit. A colposcopy was done for all women and cervical biopsy was taken if needed as a part of standard operating protocol (SOP) for the Department of Preventive Oncology, TMH. No repeat samples were collected after an interval of 30 days even if the women followed up. These women received treatment as per SOP of the Preventive Oncology Department. Women in the control arm were treated for Lab diagnosed RTIs at follow up visits.



Note- All women enrolled in the study was advised for sexual abstinence/use a barrier contraception till follow up visit**.**

Laboratory diagnosis for screen positivity [30,31]

A] Screen positive criteria for RTIs on Gram stain

1] Gram stain diagnosis of Candidiasis.

Gram stain smear showing the presence of budding yeast cells and pseudo hyphae in presence of clinical cervicitis.

2] Gram stain diagnosis of Bacterial vaginosis (BV)

The Gram stained slides were evaluated for BV using Nugent scoring system. A score of 7-10 was taken to be consistent with a diagnosis of BV.

3] Gram stain diagnosis of Gonococcal infection

Gram stain showing gram negative diplococci on endocervical smear.

4] Non-Gonococcal infection- (NGI) (surrogate marker for Chlamydial infections)

Clinical cervicitis with >10 pus cells/ per high power field on microscopic examination with no other pathogenic organism present.

B] Criteria for positive test of HPV DNA test [27]

HPV DNA was processed by Hybrid Capture 2 method which is a nuclei acid hybridization assay with signal amplification that uses microplate chemiluminescent detection. The relative light unit (RLU)/cut off ratio equal to or greater than 1, corresponding to 5000 or more copies of the virus was considered positive test.

Pharmacotherapy

All the women in case arm were given syndromic treatment on baseline as per National AIDS Control Organization guidelines (NACO).[32]

Statistical Analysis

The data was captured in SPSS-version 25. Distribution of variables under study were represented by descriptive statistics. Comparisons of baseline characteristics between women enrolled in case and the control arm were assessed by Pearson's $\chi 2$ test and Mann-Whitney U tests for categorical & continuously-scaled data respectively. Role of risk factors on presence of cervicitis were assessed by using Binary Logistic Regression. Analysis was performed by Statistical Packages for Social Sciences (Version 25.0, IBM Corp., Armonk, NY) & R version 3.5.1.

A] Analysis of primary objective

The primary objective of the study was to determine, if concomitant HPV infections with RTIs leading to mucopurulent cervicitis interfere with performance of HPVDNA HC2 testing. As per the hypothesis of the study, we expected the detection rates of cervical HPV to increase among women in the case arm after treatment of cervicitis with a course of antibiotics, if women were harbouring HPV at baseline visit. The similar changes were not expected in the control arm. The control arm would reflect the natural history of HPV in healthy women.

The present study demonstrated the case/control arm equally dynamic with regards conversions of HPV outcome status captured by HC2 test. There were fix effect/fix variable (clinical cervicitis) which the present study was powered to study its effect after treatment on the HPV DNA outcome status (outcome variable). There were conversions of HPV outcome status also seen within the control arm in a short period of time, so we expect certain uncontrolled random events /unexplained host factors to have influenced our HPV test results causing subjective variations in test results. The primary objective of the study was analysed using Linear Mix Effect Model (LME). The subjective variations within and between the study arms were accounted for, by including individual level variations as a random variable in LME model. The LME model takes into account the effect of fix variable (clinical cervicitis in the present study) and random variable (individual women in present study) on the outcome variable. (HPV status positive/not detected in present study).

B] Analysis of secondary objective

1] Prevalence of HPV infection in women with and without clinically diagnosed cervicitis was represented by Cross tabulation. Association between presence of HPV infection and clinically diagnosed cervicitis was tested by chi square test. Odds of having HPV infection

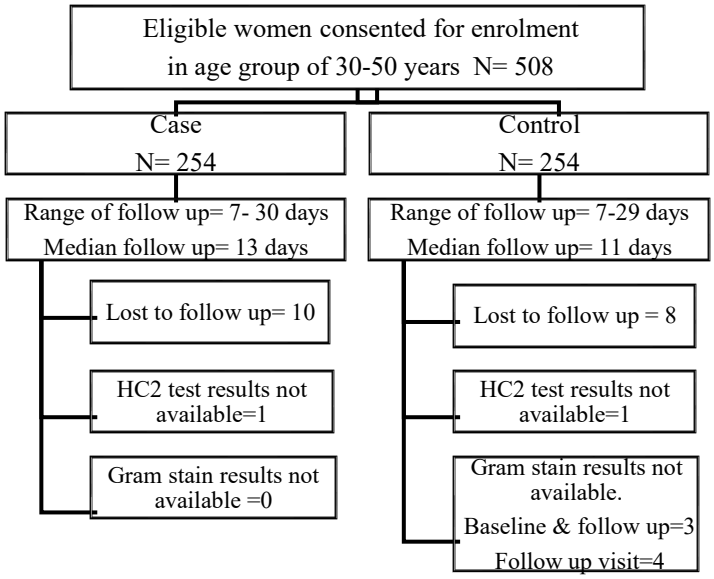
among women with cervicitis as compared to women without cervicitis was calculated by binary logistic regression.

2] To determine the prevalence of HPV infection in women with lab diagnosed RTIs, frequency with percentages were reported of women with co-infection of HPV with Candida, Bacterial Vaginosis and Non-Gonococcal infection.

Results

A] Characteristics of study population

Fig 1: Study flow chart showing baseline and follow up status of women enrolled in the study.



As shown in Figure 1, among women attending the department of Preventive oncology, sexually active non-pregnant women in the eligible age group of 30-50 years were counselled about the study and invited to participate in the study. 508 women satisfied for the eligibility criteria who consented for recruitment in the study, among these 254 women satisfying the criteria for cases were enrolled in case arm and 254 women satisfying the criteria for controls were enrolled in control arm. The range of follow up visit in case arm was 7-30 days, median follow up interval was 13 days. Among 254 women enrolled under case arm at baseline visit, 244 women followed up. 10 were lost to follow up and HC2 test result was not available for 1 woman at follow up visit.

Among 254 women enrolled under control arm at baseline visit, the range of follow up visit in this arm was 7-29 days, median follow up interval was 11 days. 246 women followed up and 8 were lost to follow up. HC2 test result was not available for 1 woman at follow up visit. Among women in the control arm Gram stain results of baseline visit were not available for 3 women and Gram stain result were not available for 4 woman at follow up due to loss of slides during transportation.

B] Descriptive analysis

In the present study majority of women were in age group of 30-40 years (66.8%) and the median age of the women enrolled in case arm was 37 years and 38 years in the control arm. There was no difference in sociodemographic and reproductive characteristics of women at baseline visit enrolled in case and control arm except clinical cervicitis in case arm versus asymptomatic women with no cervicitis in control arm (Table-1).

Table 1: Comparison of sociodemographic and reproductive characteristics of women enrolled in case and control arm at baseline Visit

Co-variates	Category	Total N=508(%)	Case Arm N=254 (%)	Control Arm N =254(%)
Age	41-50 years	169(33.3)	84(33.1)	85(33.5)
	30 to 40 years	339(66.8)	170(66.9)	169(66.5)
	Median (IQR)*	-	37 (9)	38 (8)
Marital status	Married	484(95.3)	241(94.9)	243(95.7)
	Single/separated/ widow**	24(4.7)	13(5.1)	11(4.3)
Level of education	Illiterate/primary	56(11.0)	32(12.6)	24(9.4)
	Secondary(upto12th)	204(40.2)	102(40.2)	102(40.2)
	College/university	248(48.8)	120(47.2)	128(50.4)
Religion	Hindu	471(92.7)	239(94.1)	232(91.35)
	Muslim	25(4.9)	11(4.3)	14(5.5)
	Christian/Sikh	12(2.4)	4(1.6)	8(3.1)
Occupation	House wife	326(64.2)	170(66.9)	156(61.4)
	Working women	182(35.8)	84(33.1)	98(38.6)
Monthly family income	Rs 25000 or below	255(51)	119(46.9)	136(53.3)
	Rs 25001 and above	225(45)	119(46.9)	106(41.7)
	Income not known	28(5.5)	16(6.3)	12(4.7)
Age of Menarche	Median (IQR)	-	13(1)	13(1)
Age of Marriage	Median (IQR)	-	22 (6)	23(6)
Parity	P0-P1	168(33.1)	86 (33.9)	82 (32.3)
	P2-P3	311(61.2)	150 (59.1)	161(63.4)
	P4+	29 (5.7)	18 (7.1)	11(4.3)
Menopause	Yes	6(1.2)	3(1.2)	3 (1.2)
	No	502 (98.8)	251(98.8%)	251(98.8)

*Interquartile range **Sexually active

Table 2: Comparison of contraceptive use and risk factors for reproductive tract infection among women enrolled in case and control arm at baseline Visit

Co-variates	Category	Total N=508(%)	Case Arm N=254 (%)	Control Arm N =254(%)	P value OR(CI)
Contraceptive use reported	Regular barrier user	60(11.8)	28(11)	32 (12.6)	0.413
	Other contraceptive user*	172 (33.9)	93(36.6)	79 (31.1)	
	No contraceptive users	276 (54.3)	133(52.4)	143(56.3)	
Number of sexual partners	Single	481(94.7)	234(92.1)	247(97.2)	0.0143 (1.3-.3)
	Multiple	27(5.3)	20(7.9)	7(2.8)	
Tobacco use reported	Non user	476(93.7)	238(93.7)	238(93.7)	1.000
	User	32(6.3)	16(6.3)	16(6.3)	
Previous history of RTI** treatment	No previous history of RTI treatment	330(65)	129(50.8)	201(79.1)	0.0013.6 (2.5-5.4)
	Previous history of RTI treatment reported	178 (35)	125(49.2)	53(20.9)	

*Tubectomy/Oral contraceptive pills/Intrauterine contraceptive device/Vasectomy.

** Reproductive tract infection**.**

As seen in Table 2, among the study population only 11.8% of women reported regular use of barrier contraception, 33.9% reported the use of other methods of contraception and 54.3% reported no use of contraception. There was no difference in the patterns of contraceptive use between women in the case or control arm. A total of 94.7% women in the study population reported single sexual partner while 5.3% women reported having multiple sexual partners. Among the women who reported history of multiple sexual partner, the history of multiple sexual partner was reported 3 times higher among women in case arm than in the control arm. Tobacco use were reported in 6.3% women in the study population. A total of 35% of women

reported a past history of RTI treatment. The past history of RTI treatment reported was 3.6 times higher in women in the case arm than the control arm.

Table 3: Reproductive tract infection reported on Gram stain among women at baseline visit

Category of screen positive criteria for RTIs on Gram stain	Category of women enrolled in study		
	Cases N=254 (100%)	***Control N=251 (100%)**	**Total N= 505 (100%)**
Overall positivity rates of RTIs**	239 (94)	10 (4)	249 (49.3)
Distribution of organism reported on Gram stain (criteria for screen positive for RTI infection)			
Bacterial vaginosis (Nugent score > 7)	95 (37.4)	10 (100)	105 (20.8)
Candidiasis **(**Presence of pseudohypae on the Gram stain in presence of clinical cervicitis)	15 (5.9)	0	15 (3)
Bacterial vaginosis with Candidiasis (Nugent score> 7 and presence of pseudohypae on the Gram stain in presence of clinical cervicitis)	17 (6.6)	0	17 (3.4)
Non Gonococcal Infection (Clinical cervicitis and Gram stain showing > 10 pus cells/HPF and no other pathogen reported)	112 (44.1)	0	112 (22.2)

*The above table includes women with Gram stain results available at baseline visits.

**Reproductive tract infection

The positivity rates for at least one RTI was 94% among women enrolled in the case arm versus 4% in the control arm at baseline visit. Among women in the case arm, 37.4% women had BV reported on Gram stain, 5.9% women had Candidiasis reported, 6.6% women had multiple infection of BV with Candidiasis and 44.1% women were reported to have NGI on Gram stain smear. Among 10 women diagnosed with RTI in the control arm, all women had BV reported on the Gram stain smear. The overall positivity rates of RTIs in the study population was

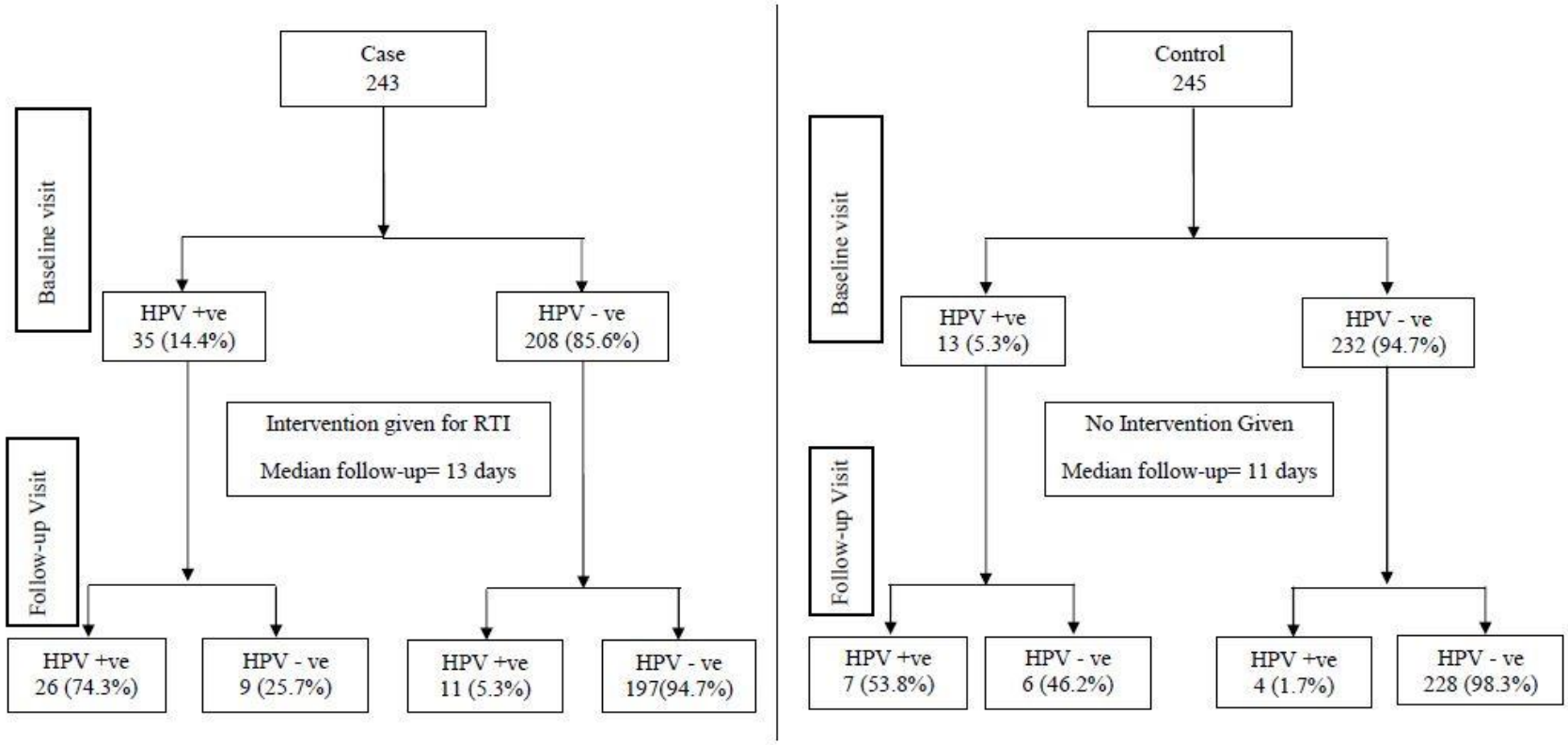
49.3%. NGI and BV were the common RTIs reported. No Gonorrhoea was reported on Gram stain smears among the women in the study population (Table-3).

The Figure 2 presents the test results of HPVDNA HC2 at baseline and follow up visit. HPV test results of baseline and follow up visits were available for 243 in the case arm and 245 in the control arm.

Among 243 women in the case arm, 14.4% (n=35) were detected HPV positive and in 85.6 % (n=208) HPV was not detected at baseline visit. Among 35 women with HPV positive at baseline visit, 74.3% (n=26) remained HPV positive and in 25.7% (n=9) HPV was not detected on follow up visit. Among 208 HPV negative women at baseline visit, in 5.3% (n=11) HPV was detected on follow up visit after treating cervicitis while 94.7% (n= 197) remained HPV negative.

Among the 245 women in the control arm, 5.3% (n=13) women were detected HPV positive and in 94.7% (n=232), HPV was not detected at baseline visit. Among the 13 women with HPV positive at baseline visit, 53.8% (n=7) remained HPV positive and in 46.1% (n=6) HPV was not detected on follow up visit. Among 232 HPV negative women at baseline visit, in 1.7% (n=4) HPV was detected at follow up visit, while 98.3% (n=228) remained HPV negative on follow up visit.

Fig 2: HPVDNA test results among women at baseline and follow up visit



Note-The above Fig includes women who followed up and had HC2 results available for baseline and follow visits

Table 4: Distribution of women according to HPV DNA test results at baseline and follow up visit

HPVDNA Conversions	Case Arm N=243(%)	Control Arm N=245(%)
Repeat test +ve (+) (+)	26(10.7)	7(2.9)
Repeat test -ve (+) (-)	9(3.7)	6(2.4)
Assumed false negative (-) (+)	11(4.5)	4(1.6)
No conversions (-) (-)	197(81.1)	228(93.1)

(+) (+) = HPV detected at baseline and follow up visit. (repeat test positive)

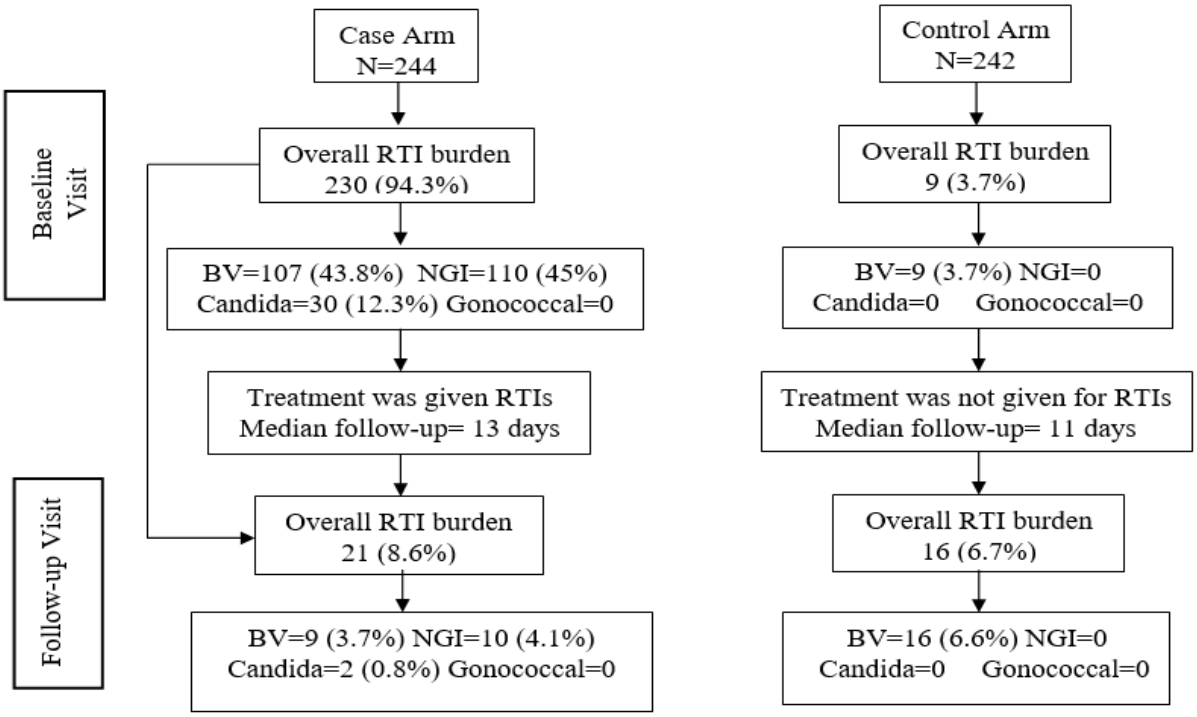
(+) (-) = HPV detected at baseline visit and not detected at follow up visit. (repeat test negative)

(-) (+) = HPV not detected at baseline visit and detected at follow up visit. (assumed false negative at baseline)

(-) (-) = HPV not detected at baseline and follow up visit. (no conversion)

Table 4 shows the overall distribution of women with HPV test results at baseline and follow up visit at median interval of 2 weeks. The overall repeat test positive result for HPV in the study population was 10.7% in case arm versus 2.9% in the control arm. The overall women with repeat test negative result were 3.7% in case arm and 2.4% in the control arm. The conversion of HPV test results from HPV not detected at baseline visit and detected at follow up visit was reported to be 4.5% after treatment of RTIs in case arm versus 1.6% among asymptomatic women in control arm with healthy cervix. 81.1% women in case arm and 93.1% women in control arm were negative at baseline and follow up visit.

Fig 3: Therapeutic cure rate for lab diagnosed Reproductive tract infection



*The above Fig includes women who followed up and had gram stains results available for baseline and follow visits to assess the therapeutic effect of syndromic treatment**.**

Among women who followed up, Gram stains results of baseline and follow up visit were available for 244 women in case arm and 242 women in the control arm. Among 244 women in case arm who received treatment for RTIs, the burden of RTIs was significantly reduced from 94.3% to 8.6%. The prevalence of BV reduced from 43.8% to 3.7%, Candidiasis reduced from 12.3% to 0.8% and NGI reduced from 45% to 4.1%. Among 242 women in control arm who received no treatment for RTIs, the prevalence of asymptomatic BV increased from 3.7% to 6.7% at follow up visit in absence of clinically evident genital infection. The women in the control arm received treatment for BV on follow up visit (Figure-3).

C] Primary Analysis

1. To study whether the presence of clinical cervicitis /or co-infections with Lab diagnosed RTIs interfere with the result of HPV DNA testing by Hybrid Capture 2 method.

Table 5: Estimates obtained through Linear Mix Effect model for primary objective

Sr.no	Parameters	Regression Estimates	Standard error	P value	Regression Estimates (Confidence interval)
1.	Intercept	0.04	0.02	0.040	0.00-0.09
2.	**Intervention (case/control)**	**0.09**	**0.02**	**0.001**	**0.05-0.14**
3.	Visit **(**BL, FU**)***	0.00	0.01	1.000	-0.02- 0.02

*BL= Baseline visit. FU= Follow up visit

There were conversions of HPV outcome status demonstrated in case and control arm. To know the true effect of our intervention on the outcome status of HPV captured by HC2 test, the subjective variability at individual level along with fix variable (clinical cervicitis in the present study) were taken into account by applying Linear Mix Effect model. The model as shown in Table 5 estimated the effect of clinical cervicitis on test results of HC2 test on follow up visit. The p value observed for the variable, interventional arm (case arm) / control arm in the model was significant (p= 0.001). The model demonstrated that intervention given for clinical cervicitis played an influencing role on modifying (increasing) the detection rates of HPV by HC2 test.

D] Analysis for secondary objectives of the study.

1. To determine the prevalence of HPV infection in women with and without clinically diagnosed cervicitis.

Table 6: Prevalence of the HPV infection among women enrolled in case and control arms at baseline visit

		Case N=254 (%)	Control N=254 (%)	Total N=508 (%)	OR (95% CI)	p value
HPV by HC2 Day-1	HPV negative	218(85.8)	241(94.9)	459(90.4)	3 (1.5%-5.9%)	0.001
	HPV positive	36(14.2)	13 (5.1)	49 (9.6)		

The overall prevalence of HPV in the present study was 9.6% at baseline visit as seen in Table 6. The prevalence of HPV infection among women in case arm was 14.2% as compared to 5.1% among women in the control arm. The risk for HPV infection was observed 3 times higher in women in the case arm as compared to women in the control arm (OR=3, CI-1.5%-5.9%).

2. To determine the prevalence of HPV infection in women with lab diagnosed RTIs at baseline visit.

Table 7: Prevalence of HPV infection in women with lab diagnosed RTIs at baseline visit

Organism reported	HPV positive N = 31	HPV negative N= 218	Total* N= 249
BV**	14 (13.3)	91 (86.7)	105(100%)
Candidiasis	0	15 (100)	15 (100%)
BV with Candidiasis	2 (11.8)	15 (88.2)	17 (100%)
NGI***	15 (13.4)	97 (86.6)	112 (100%)

*Table includes women with Lab diagnosed RTIs among women at baseline visit.

Bacterial Vaginosis, ***Non-Gonococcal infections.**

Among the total 105 BV reported at baseline visit, the prevalence of HPV infection reported was 13.3%. There was no HPV reported among women diagnosed with Candidiasis. Among multiple infection reported on Gram stain with BV and Candidiasis, the prevalence of HPV was 11.8% and prevalence of 13.4% was reported among women diagnosed with NGI (Table-7).

Discussion

The molecular HC2 test is a FDA approved test extensively validated in various cross-sectional studies worldwide. The advantage of this test as compared to other cervical cancer screening test is the reproducibility and standardization. The test identifies the women who may be at a risk of developing cervical cancer due to presence of high-risk HPV. The Multicentric cross-sectional Indian study demonstrated the potential of HC2 test to be accepted as a primary screening test.[5] The advantage of HPVDNA screening test is a good sensitivity and specificity reported from developed countries, longer screening interval if women is tested negative, the minimal resources and manpower needed for performing the test and potential of being self-collected.

Rationale - Currently there is an issue of low sensitivity of the test reported from the Indian context.[20,22] The false negative test results is a known factor to affect the sensitivity of any test. The burden of false negative test errors for HC2 test is estimated to be around 1.1.to 7.5%.[33] The common factors known to cause false negative test results of HC2 are vaginal creams and jelly,[34] improper sampling technique that may result in less cell collection,[20] improper temperature during transportation and storage that may cause denaturation of HPV DNA.[15] Since there is an issue of low sensitivity of HC2 test reported from Indian studies, the present study evaluated the probable role of RTIs resulting in false negative test errors of HC2 test. In the current study, the known factors for

false negative test errors were controlled – 1. Since there is a possibility of substances like antifungal cream, vaginal pessaries affecting the cell adequacy, women in case arm who were advised vaginal pessary as a part of RTI treatment were advised to stop the use of pessaries at least 3 days prior to second sample collection.[34] 2. The sampling technique, transportation, storage of samples was conducted as per the SOP of Microbiology department of TMH which has NABL accreditation.

To prevent new incident HPV infection during study period all women were counselled for sexual abstinence or use of barrier contraception. All women were advised to follow up within 14 days as the latency period of cervical HPV is proposed to be 2-4 weeks.[35,36,37] The study demonstrated no difference in women enrolled in case and control arm pertaining to sociodemographic data (Table 1) except clinical cervicitis in case arm. Among the risk factors for RTIs causing cervicitis, the history of multiple sexual partner and the recurrent history of RTI treatment were associated more among women in case arm than in the control arm (Table 2). The prevalence of lab diagnosed RTIs reported in case arm at baseline visit was 94% versus 4% in control arm (Table 3).

1. Repeat test positive (Persistence) with HPV on follow up visit.

The outcome of HPV test on follow up visit (follow up range 7-30 days, median follow up case arm=13 days, control arm=11 days) with that of baseline results, demonstrates some key findings of the study with respect to presence of laboratory reported RTI infections. (Fig 2). The overall persistence of HPV was reported higher among women in case arm (10.7%) than control arm (2.9%) as seen in table 4, demonstrating that existing cervicovaginal coinfections may interfere with natural history of HPV infections.[38-41]

2. Repeat test negative (Clearance) with HPV infection on follow up visit.

The study demonstrated clearance of HPV (HPV detected at baseline visit and not detected on follow up visit) within a short interval of 11-13 days. The proposed causes of these changes are - The HPV infections are transient and mostly clear within a short period of time.[42-44] Different authors reported different clearance rates at various interval of time. At present there is no consensus on definition of transient period (clearance) of HPV infection. The screening intervals,[43-48] the innate host immunity,[49-51] presence/absence of STIs/RTIs,[38-41] type specific HPV,[45,47] and viral loads[52] are known factors to affect cervical HPV clearance rates. There lies a possibility of HPV infections to be transient due to recent deposition of semen through sexual contact with an infected partner. These infections are likely to disappear if the test is repeated within a couple of weeks. The possibility of cross reactivity of probes of HC2 test leading to low RLU titers associated with weak signals cannot be entirely ruled out to be a causative factor for false positive test results.[34, 53-56]

3. HPV not detected at baseline & detected at follow up visit.

The present study demonstrated overall 4.5 % women, detected with HPV infection who were tested negative at baseline visit in case arm. These women had received treatment for mucopurulent cervicitis (table 4). The burden of RTIs in the case arm showed reduction from 94.3% at baseline visit to 8.6% on follow up visit after treatment. (fig 3) This supports the hypothesis of current study that mucopurulent discharge associated with RTIs can affect the cellularity for HC2 test. However, the control arm in the study also demonstrated 1.6 % increase in detection rates of HPV on follow up visit which was not expected.

The proposed causes for these (negative at baseline and positive on follow up) variations are, First-Cell inadequacy that may affect the test assay results, which is our proposed hypothesis.[57-59] Second-

Among the risk determinants for HPV infection the most important determinant, consistently reported in epidemiological studies is sexual activity. Infection and reinfection with HPV are strongly associated with sexual activity.[60] Third- The role of condoms in preventing new infection in cross-sectional studies is difficult to demonstrate due to transient nature of HPV infections. The limitation of the unavailability of specific data in literature review on close skin to skin contact which is a recognized mode of HPV transmission and subjective biases in reporting history of condom breakage gives rise to controversial role of condoms in completely preventing HPV infections.[35,61]

4. The impact of treatment of co-infections with HPV on HC2 test results. (Primary objective)

The present study demonstrated overall 4.5% increase in detection rates of HPV by HC2 test after treating cervicitis. In the study there were conversions of HPV outcome status (HPV detected/HPV not detected) happening within (case/control) and between the study arms at different time points. There were variables (fix variables) which we wanted to study their effect on HPV outcome status after intervention, like clinical cervicitis- (that represented case/control arm) and visits (that represented the effect of treatment on HPV detection). Second, we assume there would be factors/events which may have influenced our HPV test results leading to these variations. These factors/events needed to be accounted like -

1.The possibility of women wrongly reporting the history of condom usage.

2. Reporting of sexual partners (single/multiple) may be subjected to cultural biases.

3.Clearence due to low RLU titers.

4. Role of innate host immunity in clearance of cervical HPV.

5. Many more unknown factors that may be there due to gaps in literature pertaining to natural history of HPV.

Amidst above variations in HPV outcome status observed in the study that were assumed to be caused due to uncontrolled factors (reporting of sexual history/condom history by woman) and unassessed biological host factors (Innate immunity, other unknown factors), it becomes important to know the true effect of our intervention in improving the detection rates of HPV by HC2 test after treating cervicitis among women in the case arm (proposed hypothesis of the study). The generalised LME model was used to analysed study result. The model took into consideration the fix variable (clinical cervicitis & visits) and also the individual level subjective variations caused by the above-mentioned factors/events by including individual women as random variable in the LME model. The model demonstrated a significant role of clinical cervicitis (mucopurulent discharge) to influence the detection rates of HPV by HC2 test (Table 5).

5. Prevalence of HPV in clinical cervicitis and lab diagnosed RTIs. (secondary objective)

The prevalence of HPV infections demonstrated in present study was significantly higher in women with cervicitis (Table-6). The study also demonstrated high prevalence of HPV among women diagnosed with RTIs (BV, NGI, Candidiasis) as observed in Table 7. Cervical inflammation associated with RTIs acts as a prognostic risk factor for persistence and integration of oncogenic HPV leading to precancerous and cancerous lesion of cervix.[38, 39] The present study demonstrates the need to screen, diagnose and treat RTIs promptly in an attempt to reduce the burden of cervical cancer for the country.

Limitations of present study.

1. The possibility of cross reactivity of probes of HC2 test with low RLU titers associated resulting in false positive HPV results at baseline visit cannot be entirely ruled out.

2. The limitation of the present study was the results were not validated by PCR test for cell adequacy due to cost constrains of the HPV PCR test.

Key findings (Conclusions)

1. The study demonstrated high detection rates of HPV (14.2%) among women with clinical cervicitis (case arm) versus detection rates of HPV (5.1%) among women without cervicitis (control arm) at baseline visit.
2. The overall persistence rates of HPV in case arm (10.7%) was higher than the control arm (2.9%), thereby leading us to conclude that the presence of cervico-vaginal co-infection/s interfere with the natural history of HPV infection. We however do not rule out the possibility of other hitherto unknown variables, which could be the subject of future investigation.
3. The study demonstrated good efficacy of syndromic management in significantly reducing the burden of lab diagnosed RTIs. The study findings support syndromic treatment for RTIs for resource constrained countries.
4. The present study demonstrated the overall detection rates of HPV to improve by HC2 test by 4.5% among women treated for mucopurulent cervicitis. On controlling the biological and behaviour determinants by modelling, the study demonstrated influencing role of mucopurulent discharge associated with cervicitis on the test results of HC2 test.

   Though the study did not reach proposed statistically significant level of 10% difference in positivity rates of HPV detection after treatment of RTIs, the outcome of present study provide proof of concept that concomitant RTIs interfere with the accuracy of HPV detection by HC2 method, which was our proposed hypothesis.

References

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
3. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Meeting, World Health Organization, International Agency for Research on Cancer. Human papillomaviruses. World Health Organization; 2007.
4. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12-9.
5. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009;360(14):1385-94.
6. Inoue M, Sakaguchi J, Sasagawa T, Tango M. The evaluation of human papillomavirus DNA testing in primary screening for cervical lesions in a large Japanese population. Int J Gynecol Cancer. 2006;16(3):1007-13.
7. Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet. 2003;362(9399):1871-6
8. Petry KU, Menton S, Menton M, van Loenen-Frosch F, de Carvalho Gomes H, Holz B, et al. Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. Br J Cancer. 2003;88(10):1570.
9. Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med. 2007;357(16):1579-88.

10. Bigras G, De Marval F. The probability for a Pap test to be abnormal is directly proportional to HPV viral load: results from a Swiss study comparing HPV testing and liquid-based cytology to detect cervical cancer precursors in 13 842 women. Br J Cancer. 2005;93(5):575- 581.
11. Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M, et al. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. Br J Cancer. 2001;84(12):1616.
12. Coste J, Cochand-Priollet B, de Cremoux P, Le Galès C, Isabelle C, Vincent M, et al. Cross-sectional study of conventional cervical smear, monolayer cytology, and human papillomavirus DNA testing for cervical cancer screening. Br J Cancer. 2003;326(7392):733.
13. Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. J Natl Cancer Inst. 2006 ;98(11):765-74.
14. Kitchener HC, Almonte M, Thomson C, Wheeler P, Sargent A, Stoykova B, et al. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol. 2009;10(7):672-82.
15. Almonte M, Ferreccio C, Winkler JL, Cuzick J, Tsu V, Robles S, et al. Cervical screening by visual inspection, HPV testing, liquid-based and conventional cytology in Amazonian Peru. Int J Cancer. 2007;121(4):796-802.
16. Blumenthal P, Gaffikin L, Chirenje ZM, McGrath J, Womack S, Shah K. Adjunctive testing for cervical cancer in low resource settings with visual inspection, HPV, and the Pap smear. Int J Gynaecol Obstet. 2001;72(1):47-53.
17. Kuhn L, Denny L, Pollack A, Lorincz A, Richart RM, Wright TC. Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. J Natl Cancer Inst. 2000;92(10):818-25.
18. Salmerón J, Lazcano-Ponce E, Lorincz A, Hernández M, Hernández P, Leyva A, et al. Comparison of HPV-based assays with Papanicolaou smears for cervical cancer screening in Morelos State, Mexico. Cancer Causes Control. 2003;14(6):505-12.
19. Sarian LO, Derchain SF, Naud P, Roteli-Martins C, Longatto-Filho A, Tatti S, et al. Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV

testing as cervical screening tools in Latin America: This report refers to partial results from the LAMS (Latin American Screening) study. J Med Screen. 2005;12(3):142-9.

20. Sankaranarayanan R, Chatterji R, Shastri SS, Wesley RS, Basu P, Mahe C, et al. Accuracy of human papillomavirus testing in primary screening of cervical neoplasia: results from a multicenter study in India. Int J Cancer. 2004;112(2):341-7.
21. Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. JAMA. 2000;283(1):87-93.
22. Shastri SS, Dinshaw K, Amin G, Goswami S, Patil S, Chinoy R, et al. Concurrent evaluation of visual, cytological and HPV testing as screening methods for the early detection of cervical neoplasia in Mumbai, India. Bull World Health Organ. 2005;83:186-94.
23. NACO. Report on mid-term review of sexually transmitted infection services. December 2009. http://www.naco.gov.in/upload/Publication/STI%20RTI%20services/Other%20STI%20Material/STI%20RTI%20MONOGRAPH%20_NACP-III-.pdf.Downloaded on 28/3/2015.
24. Ray K, Bala M, Bhattacharya M, Muralidhar S, Kumari M, Salhan S. Prevalence of RTI/STI agents and HIV infection in symptomatic and asymptomatic women attending peripheral health set-ups in Delhi, India. Epidemiol Infect. 2008;136(10):1432-40.
25. Garg S, Bhalla P, Sharma P, Sahay R, Puri A, Saha R, et al. Comparison of self-reported symptoms of gynaecological morbidity with clinical and laboratory diagnosis in a New Delhi slum. Asia Pacific Population Journal. 2001;16(2):75-9.
26. Liu W, Wu EQ, Yu XH, Feng LH, Jiang CL, Zha X, et al. Detection of human papillomavirus genotypes associated with mucopurulent cervicitis and cervical cancer in Changchun, China. Int J Gynaecol Obstet. 2013;120(2):124-6
27. Malloy C, Sherris J, Herdman C. HPV/DNA testing: technical and programmatic issues for cervical cancer prevention in low-resource settings. 2000
28. Bruni L, Diaz M, Castellsagué M, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010;202(12):1789-99.
29. Sankaranarayanan R, Nene BM, Dinshaw KA, Mahe C, Jayant K, Shastri SS, et al. A cluster randomized controlled trial of visual, cytology and human papillomavirus screening for cancer of the cervix in rural India. Int J Cancer. 2005;116(4):617-23.

30. Unemo M, Ballard R, Ison C, Lewis D, Ndowa F, Peeling R. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. WHO document production services. Bull World Health Organ.Geneva.2013.
31. CDC. Disease characterised by urethritis and cervicitis. http://www.cdc.gov/std/tg2015/urethritis-and-cervicitis.htm. Downloaded on 23.5.2019.
32. NACO National guidelines on prevention, management and control of Reproductive tract infections including Sexually transmitted infections. August 2007. http://naco.gov.in/sites/default/files/National_Guidelines_on_PMC_of_RTI_Including_STI%201.pdf
33. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003 ;16(1):1-7.
34. Qiagen. hc2 HIGH-RISK TEST. Qiagen Gaithersburg, Inc.US. 2008.p 34-36.
35. Winer RL, Hughes JP, Feng Q, O'Reilly S, Kiviat NB, Holmes KK, Koutsky LA. Condom use and the risk of genital human papillomavirus infection in young women. N Engl J Med. 2006;354(25):2645-54.
36. Fernandes JV, Araujo JD, Fernandes TA. Biology and natural history of human papillomavirus infection. Open Access J Clin Trials [INTERNET]. 2013;5:1-2. doi-10.2147/OAJCT.S37741.
37. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci. 2006;110(5):525-41.
38. Williams VM, Filippova M, Soto U, Duerksen-Hughes PJ. HPV-DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress. Future Virol. 2011 ;6(1):45-57.
39. Castle PE, Giuliano AR. Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients—assessing their roles as human papillomavirus cofactors. J Natl Cancer Inst Monogr. 2003;2003(31):29-34.
40. King CC, Jamieson DJ, Wiener J, Cu-Uvin S, Klein RS, Rompalo AM, et al. Bacterial vaginosis and the natural history of human papillomavirus. Infect Dis Obstet Gynecol. 2011;2011:p8.
41. Silins I, Ryd W, Strand A, Wadell G, Törnberg S, Hansson BG, et al. Chlamydia trachomatis infection and persistence of human papillomavirus. Int J Cancer. 2005;116(1):110-5.
42. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst. 2011 ;103(5):368-83.

43. Rodríguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle PE, et al. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008;100(7):513-7.
44. Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM, ALTS (Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study) Group, Plummer M. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis. 2007;195(11):1582-9.
45. Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii Human Papillomavirus Cohort Study. Cancer Res. 2008;68(21):8813-24.
46. Dalstein V, Riethmuller D, Prétet JL, Le Bail Carval K, Sautière JL, Carbillet JP, et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int J Cancer. 2003;106(3):396-403.
47. Molano M, van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am J Epidemiol. 2003;158(5):486-94.
48. Rosa MI, Fachel JM, Rosa DD, Medeiros LR, Igansi CN, Bozzetti MC. Persistence and clearance of human papillomavirus infection: a prospective cohort study. Am J Obstet Gynecol. 2008;199(6):617-e1.
49. Moscicki AB, Schiffman M, Kjaer S, Villa LL. Updating the natural history of HPV and anogenital cancer. Vaccine. 2006;24:S42-51.
50. Mariani L, Venuti A. HPV vaccine: an overview of immune response, clinical protection, and new approaches for the future. J Transl Med. 2010;8(1):105.
51. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30:F55-70.
52. Kim JW, Song SH, Jin CH, Lee JK, Lee NW, Lee KW. Factors affecting the clearance of high-risk human papillomavirus infection and the progression of cervical intraepithelial neoplasia. J Int Med Res. 2012;40(2):486-96.

53. Gillio-Tos A, De Marco L, Carozzi FM, Del Mistro A, Girlando S, Burroni E, et al. Clinical impact of the analytical specificity of the hybrid capture 2 test: data from the New Technologies for Cervical Cancer (NTCC) study. J Clin Microbiol. 2013;51(9):2901-7.
54. Preisler S, Rebolj M, Ejegod DM, Lynge E, Rygaard C, Bonde J. Cross-reactivity profiles of hybrid capture II, cobas, and APTIMA human papillomavirus assays: split-sample study. BMC cancer. 2016;16(1):510.
55. Sargent A, Bailey A, Almonte M, Turner A, Thomson C, Peto J, et al. Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. Br J Cancer. 2008;98(10):1704.
56. Castle PE, Schiffman M, Burk RD, Wacholder S, Hildesheim A, Herrero R, et al. Restricted cross-reactivity of hybrid capture 2 with nononcogenic human papillomavirus types. Cancer Epidemiol Biomarkers Prev. 2002;11(11):1394-9.
57. Jastania R, Geddie WR, Chapman W, Boerner S. Characteristics of apparently false-negative digene hybrid capture 2 high-risk HPV DNA testing. Am J Clin Pathol. 2006;125(2):223-8.
58. Wheeler CM, Greens CE, Becker TM, Hunt WC, Anderson SM, Manos MM. Short term Fluctuation in the Detection of Cervical Human Papillomavirus. Obstet Gynecol. 1996;88(2): 261-8.
59. Van Ham MA, Melchers WJ, Hanselaar AG, Bekkers RL, Boonstra H, Massuger LF. Fluctuations in prevalence of cervical human papillomavirus in women frequently sampled during a single menstrual cycle. Br J Cancer. 2002;87(4):373.
60. Trottier H, Ferreira S, Thomann P, Costa MC, Sobrinho JS, Prado JC, et al. Human papillomavirus infection and reinfection in adult women: the role of sexual activity and natural immunity. Cancer Res. 2010;70(21):8569-77.
61. Christopher A. Hearing addresses condoms for HPV prevention. J Natl Cancer Inst. 2004;96(13):985.

LIST OF ABBREVIATIONS

STIs	Sexually transmitted infections
RTIs	Reproductive tract infections
HPV	Human Papillomavirus
VIA	Visual inspection with acetic acid
HC2	Hybrid Capture 2 test
PCR	Polymerase Chain Reaction
RCT	Randomised control trial
CIN	Cervical intraepithelial neoplasm
CIN2+	Cervical intraepithelial neoplasm grade 2 /Carcinoma in situ
WHO	World Health Organization
NACO	National AIDS Control Organisation
SOP	Standard operating protocol
ASR	Age Standardised Rates
PBCR	Population based cancer registry
LMIC	Low middle income countries
BV	Bacterial Vaginosis
T.Vaginalis	Trichomonas Vaginalis
NGI	Non-Gonococcal infection
NABL	National accreditation board for testing and calibration laboratory
PMN/HPF	Polymorphonuclear leucocytes/High power field
RLU	Relative light index
TMH	Tata Memorial Hospital
WIA	Cancer Institute Adyar- Chennai
PGIMER	Postgraduate Institute of Medical Education and Research- Chandigarh
KMIO	Kidwai Memorial Institute of Oncology
RCC	Regional Cancer centre- Thiruvananthapuram
AMC	Assam Medical college
BBCI	Dr. Bhubaneswar Borooah Cancer Institute
PID	Pelvic Inflammatory Disease
OPD	Out Patient Department

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Cervical cancer is a global health issue among women in reproductive age group. It is the fourth most common cancer reported among women globally and second most common cancer reported among Indian women.[1,2] As per Globocan 2018 estimates, 569,847 new cervical cancer cases and 311365 cervical cancer deaths were reported worldwide. Substantial burden of cervical cancer is contributed by developing countries.[1,2] The social determinants like illiteracy, lack of awareness to the fact that cervical cancer is a preventable disease and the lack of priority given to health issues by women in developing countries contributes to high burden of cervical cancer reported from these countries. At national level, factors contributing to high burden are lack of good quality screening programs, access to these services and inadequate coverage of population which are crucial components for effective cervical cancer screening programmes.[3] Among the South Asian countries, India is reported to have huge burden of cervical cancer with 96,922 incident cases and 60,078 deaths cases reported as per Globocan 2018 estimates.[1,4] India contributes 23% of the global burden of cervical cancer.[5] Most Indian women present with advanced stages of cancer in hospitals which is associated with low cancers survival rates. The 5-year survival rates for cervical cancer for India, using population based cancer registry (PBCR) data was reported to be 46% which was comparably very low when matched to other Asian countries.[6] The high incidence and low survival rates of cervical cancer reported from India emphasizes on the need of prompt diagnosis and treatment of cervical cancers with cost effective screening tools and treatment regimes. A cost-effective screening tool in terms of high sensitivity, necessitating less frequent screening rounds and achieving adequate population coverage is a pre-requisite for success of cervical cancer screening program at a national level.

Human papilloma virus (HPV), a sexually transmitted infection, is the necessary cause for cervical cancer worldwide.[4,7] At present, there are about 15 HPV types recognized to be cancer-causing (oncogenic types) with type 16 and18 contributing to 70% of cervical cancer burden globally.[4,8,9] There is a strong causal association between persisting genital tract infection with oncogenic HPV and gradual progression of the infection to invasive cervical cancer.[7,10] As per natural history of HPV, the cervical cancer has a recognised multistage ranging from- a) Acute infection with oncogenic HPV. b) HPV being transient infection majority of women clear the infection spontaneously within 1-2 years and only 5-10% women may have persistent HPV infection. c) The women with persistent infection with oncogenic HPV progress to precancerous lesions (cervical intraepithelial neoplasm). d) If not diagnosed and uninterrupted by intervention, 50% of severe precancerous lesion progress to invasive cancer. The progression of HPV infection to cancerous lesions happens over a decade.[7,11] Since cervical cancer is a multistage disease, with a long and well understood natural history gives a substantial window of opportunity for screening. The natural history can be influenced by co-factors like parity, tobacco use, STI/RTIs and innate immunity plays an important role in clearance/ persistence of infection.[12-17] Cervical cancer screening identifies woman at an asymptomatic stage who may have the risk of developing cervical cancer if not detected and treated.

The Pap cytology, Visual inspection with acetic acid (VIA), HPVDNA based molecular test are currently available screening test for cervical cancer. Screening with Pap cytology has decreased the cervical cancer mortality among developed countries,[18,19] but in Low Middle Income Countries (LMIC) similar results are not evident for various reasons. It is difficult to implement cytology-based screening program in LMIC mainly due lack of awareness among women, uneven distribution of health care facilities, lack of infrastructure in terms of laboratory, a trained cytologist to interpret the test reports with quality control measures in

place and trained staff to conduct the test.[20,21] Pap test has been reported to have suboptimal sensitivity.[22,23]

Screening with VIA appears the most feasible and cost-effective option for LMICs, with advantages of results being available immediately and the test showing good performance even with a trained non-clinical provider. RCTs from India has evaluated the effectiveness of VIA in significant reduction of the mortality of cervical cancer.[24,25] The short comings of screening programs with VIA is its high false positivity test results, leading to increased burden for further diagnostic investigations in the health care system.[24,25,26,27,28] The short-term screening intervals needed by cytology or VIA based screening can contribute to logistic challenges especially in developing countries in terms of surveillance.[29,30] The direct detection of HPV in cervical specimen by a molecular test can offer an alternative for cytology/VIA based screening. Among the molecular HPVDNA tests, the Hybrid capture 2 test (HC2) has been extensively evaluated in various settings across the world. The test identifies 13 recognised oncogenic viruses involved in cervical carcinogenesis. It becomes easier to implement HPV based screening program in countries due to the high sensitivity of the test reported and needing longer screening interval for the women tested negative. This proves to be cost-effective, in terms of lesser screening rounds needed by HPVDNA based screening. The robust randomized control trial (RCT) from India, randomized around 131,746 women in the age group 30-59 years into four groups to undergo different cervical cancer screening modalities namely HPVDNA test by HC2 method, Pap cytology, VIA versus no screening. The study demonstrated, single round of HC2 test based screening to significantly reduce the cervical cancer mortality by 48% at the end of 7 years.[31] Developing country like Mexico failed to demonstrate decrease in cervical cancer mortality using Pap smear as a screening test. The country later evaluated HC2 test versus Pap cytology to test the best screening modality for their country. The study demonstrated a sensitivity of 93.1% for clinician collected HC2 test

as compared to 59.4% for Pap cytology to diagnose CIN2+ lesions. The country later introduced HC2 test as a primary screening modality for women aged 35–64 years for cervical cancer and HPV vaccination for girls aged 9–12 years in their national immunization program in 2008.[21,32,33,34]

The above findings are encouraging since it demonstrates that a simple and reliable HPV DNA test (HC2) which is now available in middle- and low-income countries has a potential to be accepted as primary screening test in the future. At present HC2 molecular test is a proven triage test for women with atypical cytology.[35] Among the developed countries, United states, European countries, Australia, Netherlands are adopting HPV based screening in their national screening program.[36,37,38]

The population based cross sectional studies from developed countries[39-47] like Switzerland, France, UK (HART study), Japan, Great Manchester (ARTISTIC trial) Germany (Montreal & St John's church) and Italy evaluated HC2 test to diagnose cervical intraepithelial neoplasia grade -2 & above lesions (CIN2+). These studies demonstrated HC2 test sensitivity of 90% to 100% with an average of 95%.

Similarly, the population based cross sectional studies from developing countries[32,48-53] like Peru, Latin America, Zimbabwe, South Africa, India, Mexico and Costa-Rica also evaluated HC2 test to diagnose CIN2+ lesions. In these studies, HC2 test demonstrated sensitivity of 50% to 91% with an average of 79%. Among the developing countries cross-sectional study from India which evaluated test characteristics of HC2 in detecting CIN-2+ lesions have shown substantially low sensitivity of 68.2%.[52]

There is a statistically significant difference (p value = 0.003) of sensitivity of HC2 test between the developed and developing countries to determine CIN2+ lesions while there is no

difference in the specificity. The similar finding of sensitivity of HC2 test from various countries to diagnose CIN2+ lesions were also reported by M. Arbyn.[54]

Various reasons for low sensitivity from developing countries have been discussed like fluctuations in temperature during transportation that can lead to denaturation of HPVDNA, improper sampling technique of collecting cervical HPVDNA, verification biases, prevalence of HPV in a particular geographical area and availability of HC2 probes of the common HPV types present in that given area.[32,48-53]

A meta-analysis conducted on a million women with normal cytological findings across the world to estimate prevalence of cervical HPV using standard HPVDNA test kits (PCR/HC2), the author mentioned that even though the prevalence rate of cervical HPV were lower for Southern Asian region mainly India (7.9%), the incidence rates of cervical cancer was reported high.[55] Similar findings was reported by de Martel et al.[4] This reflects the possibility of missing a huge number of HPV infections.

Worldwide, the major cause of STI/RTIs leading to cervical inflammation (cervicitis/cervicovaginitis) reported are Gonorrhoea, Chlamydia, Trichomoniasis, Candidiasis, Bacterial Vaginosis and Mycoplasma genitalium.[56,57] World Health Organisation (WHO) estimates 340 million new STIs to occur globally each year among men and women in the age group of 15 – 49 years, which is preventable by screening.[57] Among developing countries, the highest number of STIs are reported from Africa and Southeast Asian countries.[57] The burden of STIs/RTIs are expected to be high among middle- and low-income countries as these countries lack development of quality care and treatment services due to resource constraints.[58]

The accurate data regarding the prevalence of STIs/RTIs is lacking for India, though the point prevalence of the disease is estimated around 30 million.[59] For a developing country like India,

the burden of RTIs among women is expected to be higher than reported due to illiteracy, ignorance, poor knowledge of contraception, cultural norms, limited and ineffective health care facilities for screening RTIs and poor access to health care facilities, if available.[59,60,61] The most common bacterial RTIs reported from India is Bacterial Vaginosis (BV) followed by Candidiasis.[61] There is decreasing trends of bacterial STI and increasing trends of viral STIs reported from India.[62,59,63] Among the viral STI, HPV is a cause of concern due its association with cervical cancer. Studies have shown mucopurulent cervicitis caused due to bacterial STIs / RTIs to be associated with high prevalence of HPV infection.[64,65,66,67] There is evidence of association of BV, cervicitis and uterine cervical HPV infection reported.[68-71] Till date there are limited studies from India addressing the above issues in general population. The only community-based study conducted to our knowledge by Parmar et al in rural district of Thane, India, to estimate the prevalence of STI/RTIs among 415 women in the reproductive age group, the author reported prevalence of clinical cervicitis to be 55.2% in the general population.[72]

There has been a concern of the blood and mucopurulent discharge associated with cervical inflammation due to RTIs interfering with the sensitivity of screening test for cervical cancer.[73,74] Liu W et al conducted a study among Chinese women to detect the prevalence of HPV genotypes among three groups of women, group 1- women with mucopurulent cervicitis, group 2- healthy women and group 3- women with invasive cancer. The author reported a 10% higher failure rates to extract HPV DNA among group of women with mucopurulent cervicitis as compared to other two groups.[66] Since the potential of any primary screening test to be adopted by a country depends on the sensitivity of the test, it becomes important to address the issue of low sensitivity of HC2 test from Indian context.[52,24] Among the molecular based HPVDNA tests, HC2 test is extensively evaluated in various settings and is considered as a gold standard test to diagnose HPV infection. The limitation of the test is, no inbuilt mechanism to monitor cell adequacy. The test result is interpreted as ‘detected’ when any one

of the 13 oncogenic HPV types is present with viral copies above 5000 in cervical sample or 'not detected'. The 'not detected' test result opens a debate if the sample has less amount of HPVDNA viral copies or its truly negative with no HPV infection.[75] The present study evaluates the influence of RTIs associated with mucopurulent discharge as a probable cause of false negative results of HC2 test.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

2.1. Burden of disease - Cervical Cancer.

2.1.1. Burden of cervical cancer - Global [1]

Cancer of the uterine cervix is the fourth most common cancer globally with 569,847 new cases and 311,365 deaths respectively reported in 2018. (Fig 2.1.1) The substantial burden of cervical cancer is contributed by African countries followed by Melanesia. (Fig 2.1.2)

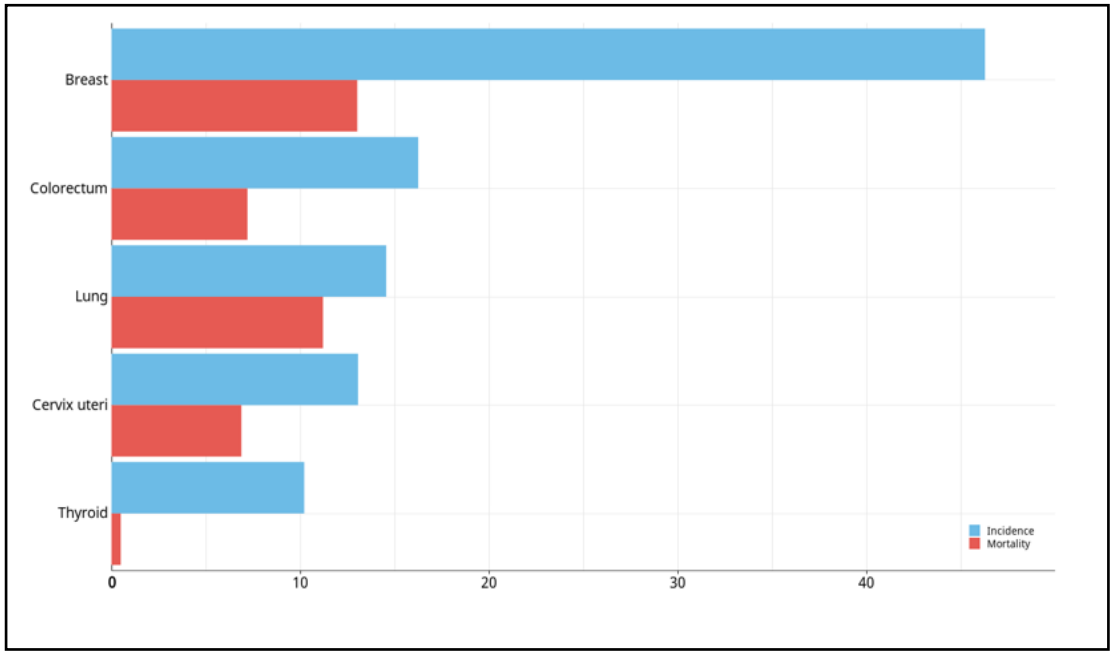


Fig 2.1.1. Age standardized incidence and mortality rates of cancers women reported globally, among all ages. (Source Globocan 2018)

The high-income countries have qualitative and effective cancer screening services in place, while the middle- and low-income countries (developing countries) lack these services. Lack of adequate health care infrastructure for screening common cancers and inaccessibility to such facilities contribute to high burden of cervical cancer cases reported from the developing countries. High burden of cervical cancer is also contributed by social determinants like low literacy levels, lack of awareness regarding symptoms of cervical cancer, availability and accessibility of screening services and low priority given to health issues by woman in

developing countries. These factors also contribute to inadequate population coverage for cervical cancer screening, which is a crucial component for cervical cancer screening programme to be effective. A population-based survey conducted across 57 countries reported low coverage of around 19% for cervical cancer screening in developing countries (ranging from 1% in Bangladesh to 73% in Brazil) compared to 63% in developed countries. The author commented that older and poor women, who are at the highest risk of developing cervical cancer, are least likely to be screened for the disease.[3]

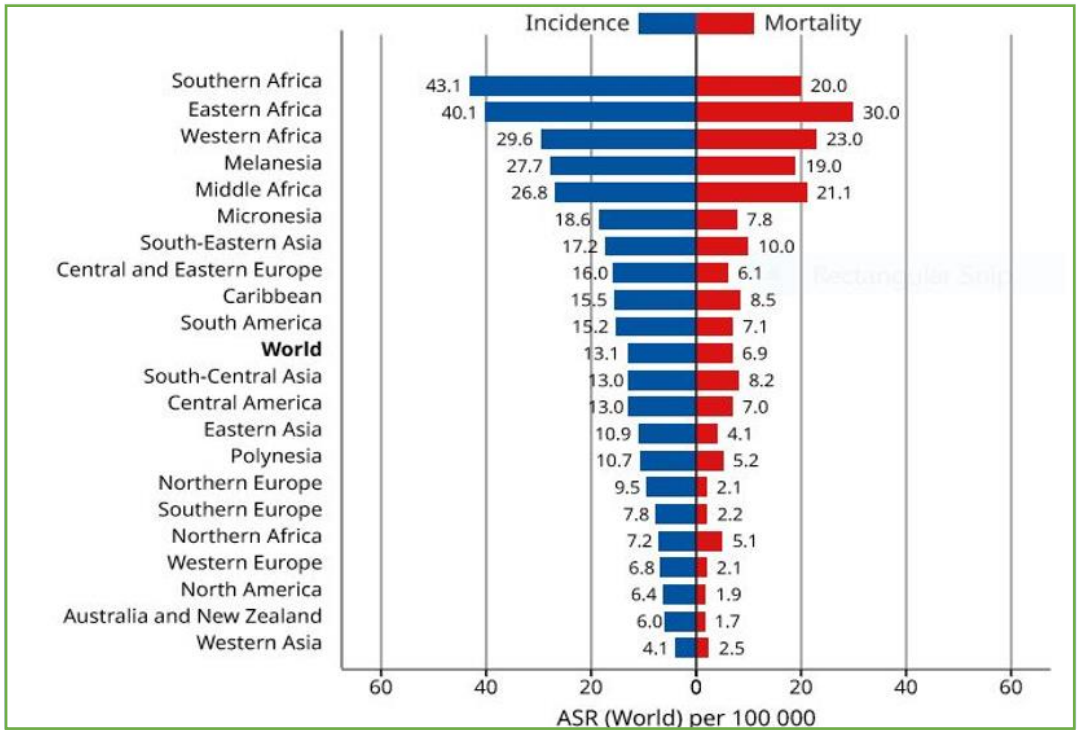


Fig 2.1.2. Age Standardized (world) incidence and mortality rates for cervical cancer among countries. (Source Globocan 2018)

2.1.2. Burden of cervical cancer – India [1]

The most common cancers reported among Indian women is breast cancer followed by cervical cancer (Fig 2.1.3).

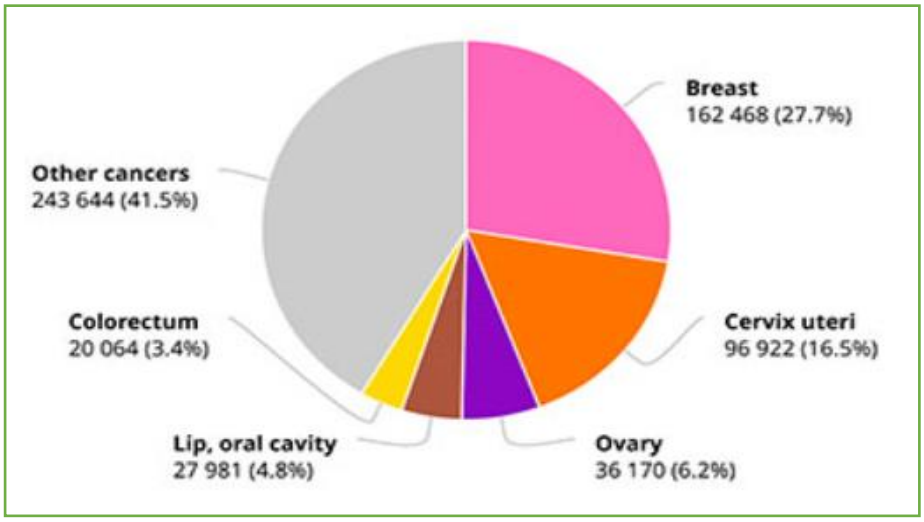


Fig 2.1.3. Number of new cancer cases reported for Indian women, all ages (Source Globocan 2018)

When compared to developed countries, India has a huge burden of cervical cancer (Fig 2.1.4).

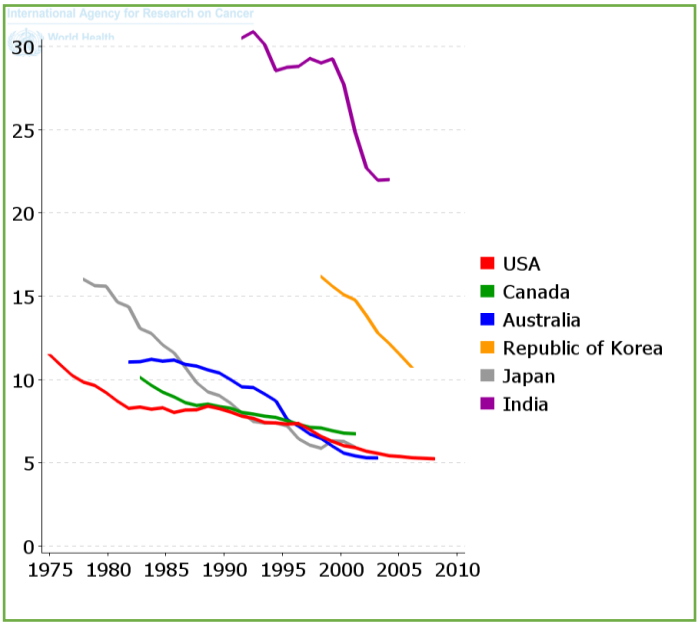


Fig 2.1.4. Trends in incidence of cervical cancer in selected countries – Age-standardised rate[w] per 100,000 (Source Globocan 2012)

Among the South Asian countries, Nepal reports the highest Age Standardized incidence of cervical cancer - 21.5 per 1,00,000 followed by India and Bhutan viz. 14.7 and 14.4 per 1,00,000 respectively (Fig-2.1.5).[1] India contributes to 23% of global cervical cancer burden.[5]

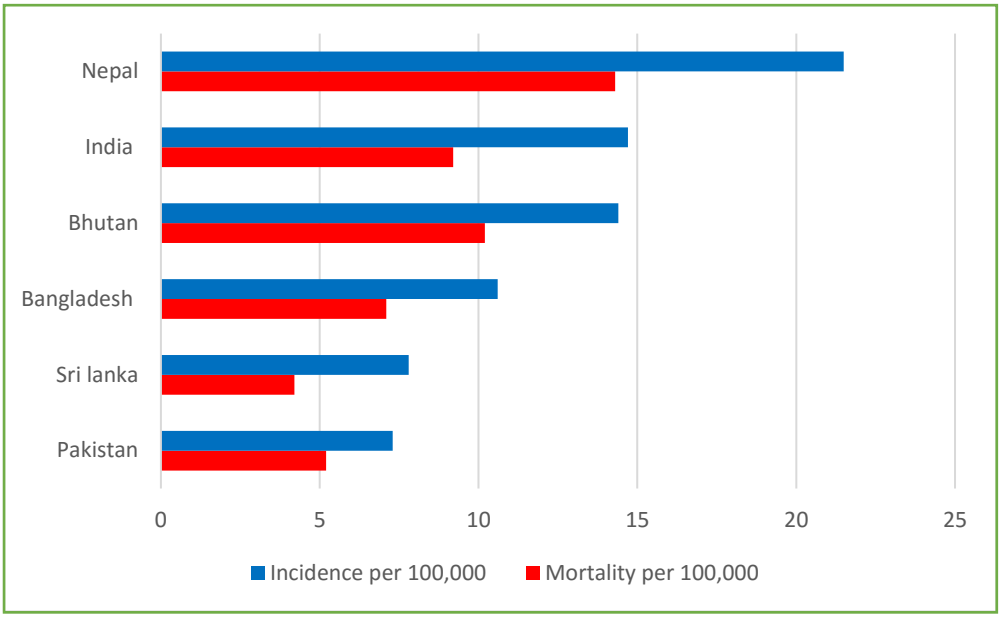


Fig 2.1.5. Age standardized incidence and mortality rates for cervical cancer reported from South East Asian Countries. (Source Globocan 2018)

Estimated incidence of cervical cancer in India. [1]

CANCER	NUMBER	%	ASR per 100000
Cervical cancer	96,922	8.4%	14.7

Estimated mortality of cervical cancer in India.[1]

CANCER	NUMBER	%	ASR per 100000
Cervical cancer	60,078	7.7%	9.2

2.1.2.a. Burden of cervical cancer in India across Population based cancer registries

In the metropolitan cities of India, breast cancer is the commonest cancer reported, whereas cervical cancer remains the most common cancer encountered mainly in rural India. Among the population-based cancer registries the incidence of cervical cancer varies among different regions in India (Fig 2.1.1). The highest incidence rates of cervical cancer are being reported from north eastern regions; namely Papumpare District (AAR-30.2), Aizawl District (AAR-28.0) and Mizoram State (AAR-23.1). Apart from the lack of quality health-care facilities available in these states, the traditional risk factors such as consumption of tobacco and smoking act as co-factors contributing to the high incidence of cervical cancer reported.[76]

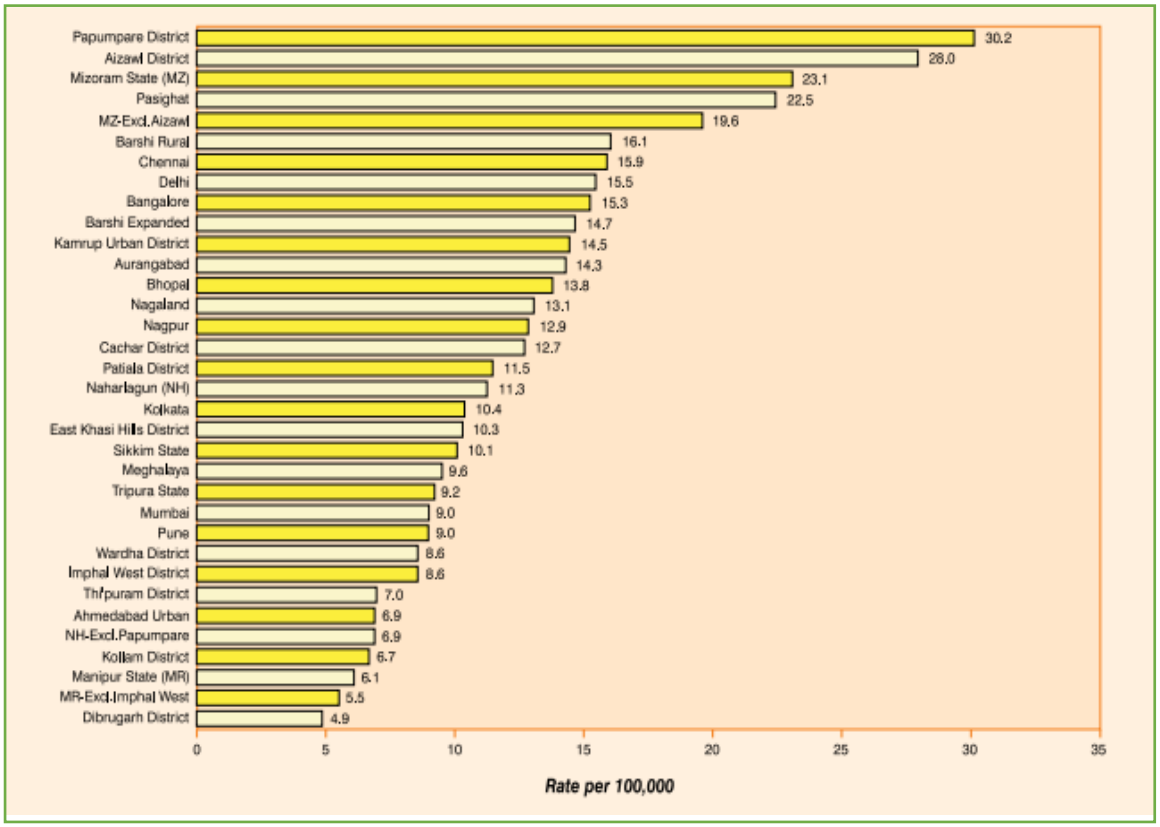


Fig 2.1.6. Comparison of Age adjusted cervical cancer incidence and patterns across population-based cancer registries in India. (Source www.ncdirindia.org/All_Reports/PBCR_REPORT_2012_2014/index.htm. Accessed on 4.4.19)

Since 1982, we witness a declining trend of cervical cancer incidence rates across all population-based cancer registries in India. However, it still remains a major health issue among Indian women. Among the metropolitan cities the highest cervical cancer burden is reported from Chennai, followed by Delhi and Bangalore (Fig 2.1.7).

The high cervical cancer incidence and mortality rates in India can be attributed to factors such as low socioeconomic status, low literacy levels, social stigma associated with the disease, nutritional deficiency, poor genital hygiene, limited contraceptive knowledge making women prone to repeated pregnancies and recurrent STIs.[5,77-80] It has been postulated that individuals with low income are more likely to engage in high risk sexual activity. Cervical cancer is a social disease in true sense.

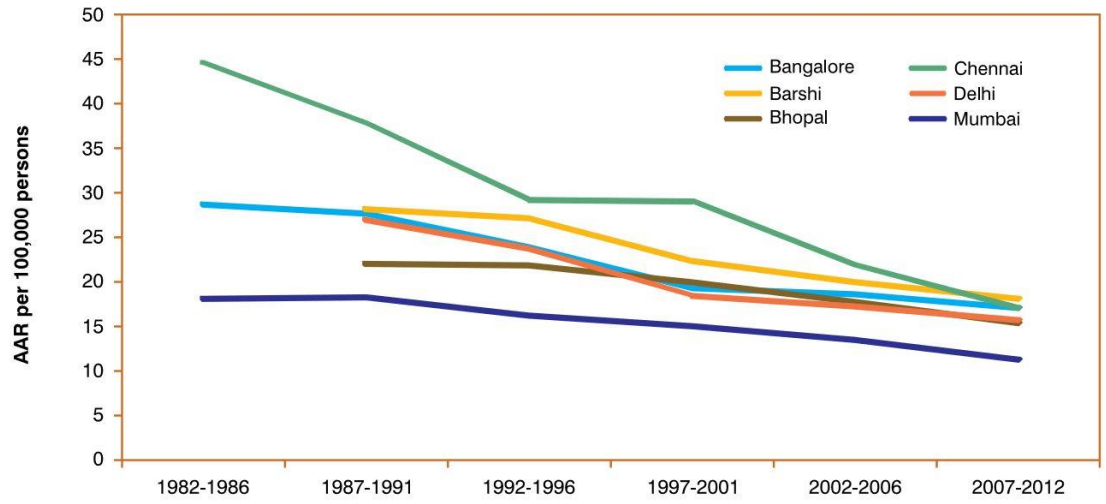


Fig 2.1.7- Incidence of cervical cancer among Indian population-based cancer registries (Source www.ncdirindia.org/All_Reports/PBCR_REPORT_2012_2014/index.htm. Accessed on 4.4.19)

High mortality due to cervical cancer, reported from India, is also a reflection of health seeking behaviour of Indian women. Most women are not aware of signs and symptoms pertaining to cervical precancer and cancer. Hence, most women present with advanced stages of cancer to

the health care system. This in turn is associated with low cancer survival rates. A study conducted to analyse survival data of cervical cancer among 12 countries in Sub-Saharan Africa, Central America and Asia using data from population-based cancer registries, reported only 46% five-year survival among Indian women. This is comparably lower than rates in other Asian countries (Fig 2.1.8). The key factors responsible for survival rates of cervical cancers are availability and accessibility of quality health care services.[6]

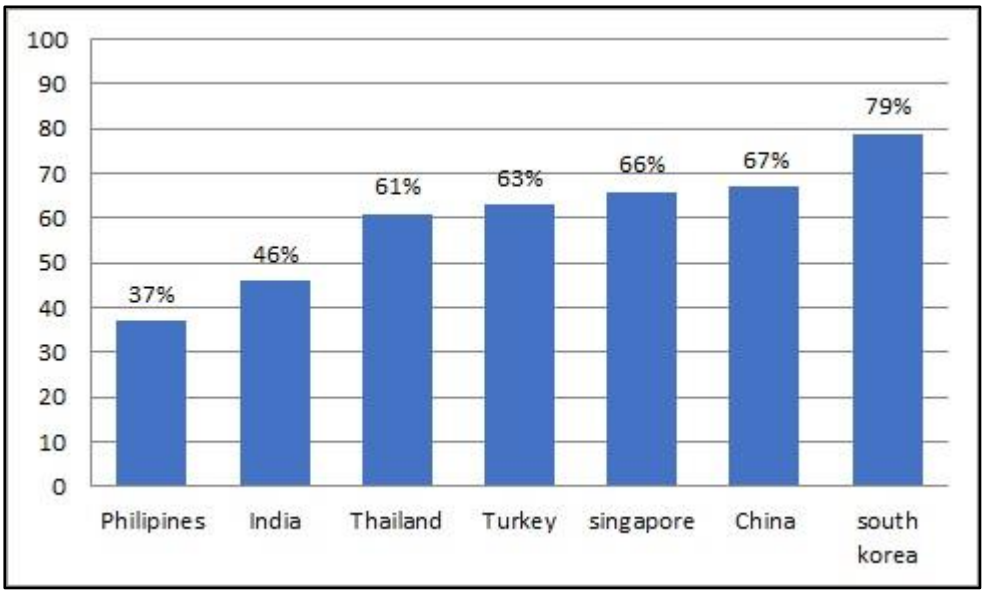


Fig 2.1.8. Median percentage of 5-years Age Standardised Relative Survival for cervical cancer among selected Asian countries. (Source Sankaranarayanan R, *et al.* Lancet Oncol. 2010.)

There also lies the possibility of incidence and mortality figures to be underestimated in India due to issues related to heterogeneous sources of data collection, lack of linkage systems at national levels, lack of effective referral system, improper recording of cause of death and poor quality of data regarding the follow up of patients in hospitals.[6,81]

2.1.2.b. Burden of cervical cancer in India across Hospital based cancer registries

Among the hospital-based cancer registries (HBCRS) of India, cervical cancer remains among the top two common cancers reported across all hospital registries. Among the trends observed for cervical and breast cancers during the period from 2007-2014 in India, breast cancer remained leading site of cancer followed by cervical cancer in TMH-Mumbai and AMC-Dibrugarh registries. WIA-Chennai, PGI-Chandigarh and BCCI- Guwahati reported a change

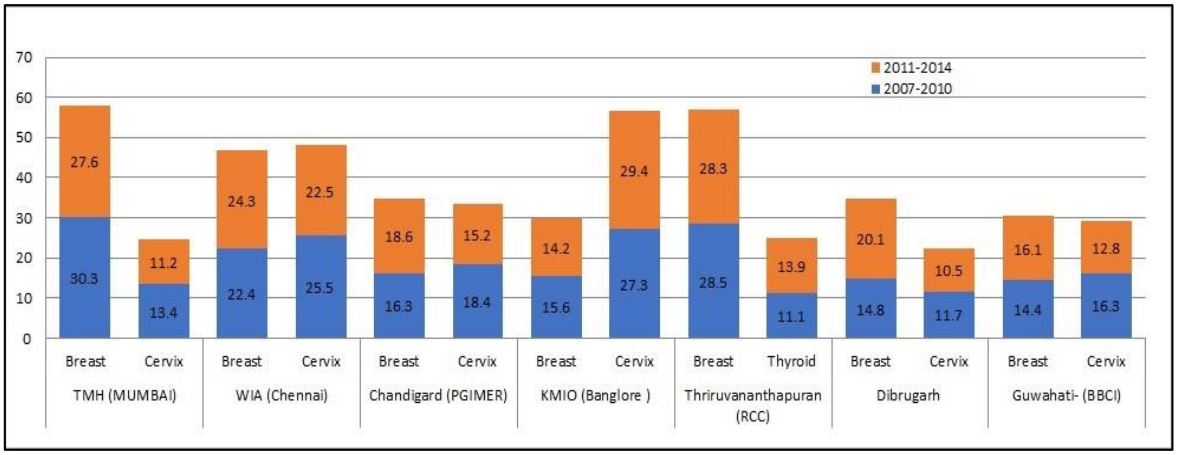


Fig 2.1.9. The trends of common cancer reported across the hospital-based cancer registries in India from 2007- 2014. (Source www.ncdirindia.org/All_Reports/HBCR_REPORT_2012_2014/index.htm. Assessed on 4.4.19)

in trends, where in cervical cancer was the leading site reported during the period of 2007-2010, while breast cancer was reported as leading site during the period from 2011-2014. Cervical cancer remained the leading site of cancer for KMIO-Bangalore for above mentioned period. For RCC-Thiruvananthapuram, breast cancer remained leading site followed by thyroid cancer during the period of 2007-2014. Cervical cancer is reported to be third most common cancer reported from this registry (Fig 2.1.9).

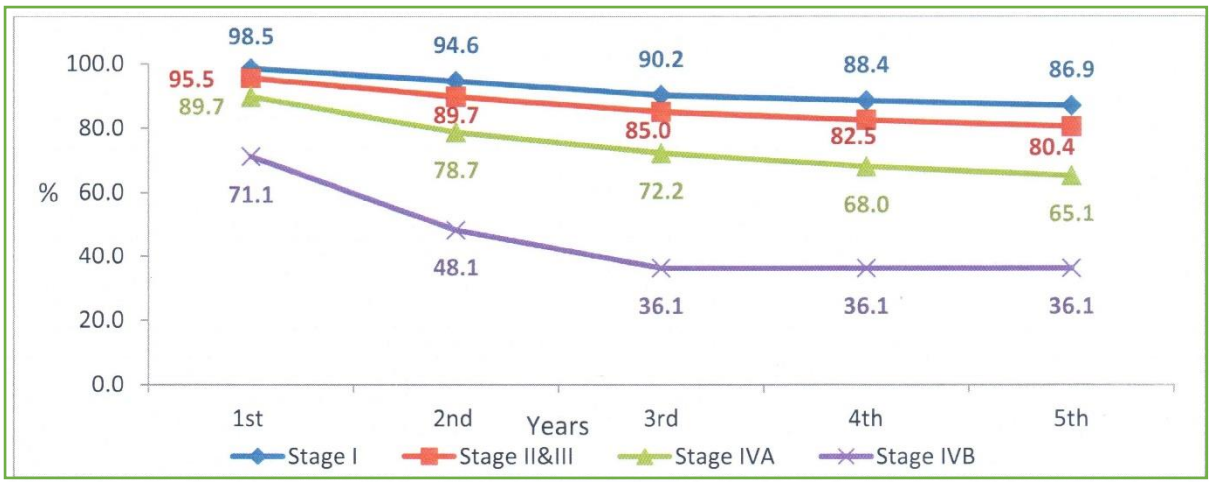


Fig 2.1.10. Stage presentation of cervical cancer at Tata Memorial Hospital. (Source A Handbook of Department of Medical records, Biostatistics and Epidemiology. Tata Memorial Centre. Feb 2019)

Cervical cancer is preventable by screening and treatable if detected early. In Indian majority of cervical cancer cases present with loco-regional disease, leading to low cervical cancer survival rates (Fig 2.1.10). Factors contributing to low cancer survival rates apart from ignorance and social stigma are, limited access to quality treatment and treatment failures due to treatment gaps.[82]

2.2. Epidemiology of Cervical Cancer

2.2.1. Human papilloma virus and cervical cancer

Among infectious agents causing cancers the most common infectious agent reported is H. Pylori followed by HPV.[83] HPV causes cervical, anal, vulvar and vaginal cancers among women. Globally, in 2012, 530,000 cervical cancer cases reported were 100% attributed to be caused by HPV.[4] African countries reported the highest prevalence of HPV attributed cancer cases. (Fig 2.2.1)

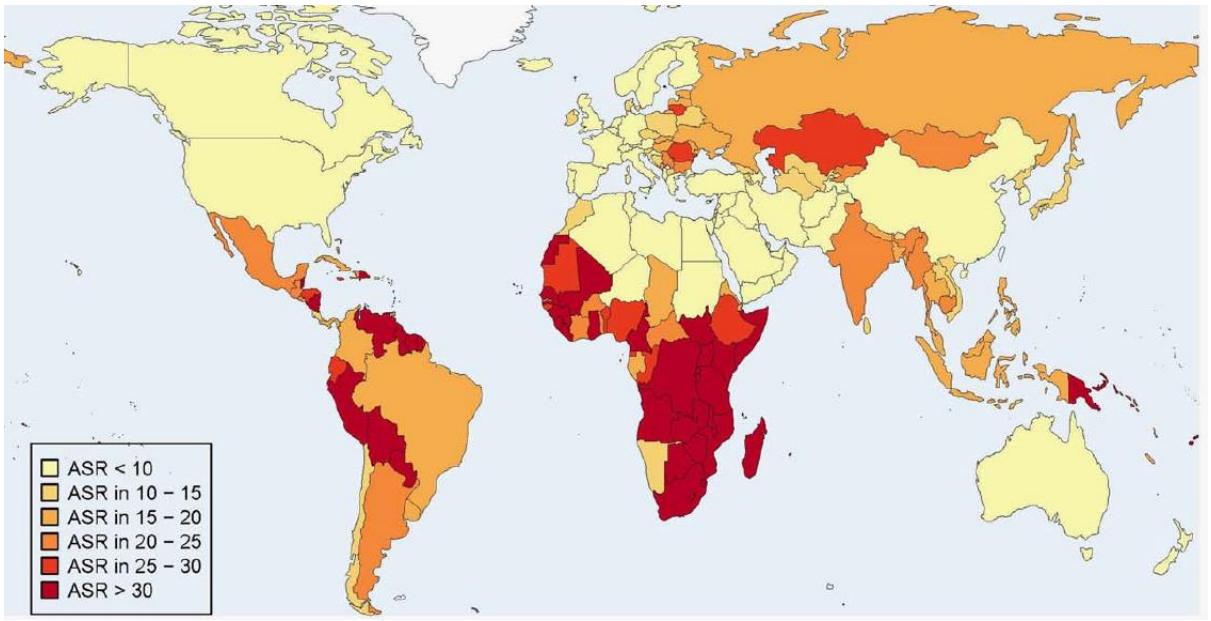


Fig 2.2.1. Worldwide burden of cervical cancer (Age Standardized Incidence rates per 100,000) attributed to HPV by country (Source de Martel C, et al. International journal of cancer. 2017)

There is sufficient evidence from epidemiological studies regarding causal role of persistent oncogenic HPV infection to be the main cause for cervical cancer. 99.7% of cervical cancers are now believed to be caused by a sexually transmitted virus, namely HPV.[7,84,85] Dr. Harald Zur Hausen received the noble prize in 2008 for isolating oncogenic HPV strains and

explaining the process of cervical carcinogenesis associated with the virus. Among the HPV subtypes, type 16 and 18 contribute to 70% of cervical cancer burden worldwide.[4]

2.2.2. Natural history of cervical cancer (Fig 2.2.2)

The prevalence of cervical HPV infection is highest among the youngsters with onset of sexual activity [i.e. around 15- 20 years of age].[55] Cervical HPV is a transient infection and majority of the women clear this infection spontaneously within 1- 2 years. Only 5-10% of those women who harbour persistent infection with oncogenic HPV type may develop pre-cancerous lesions and less than 50% of severe precancerous lesions progress to invasive cancer if uninterrupted by intervention.[11,86]

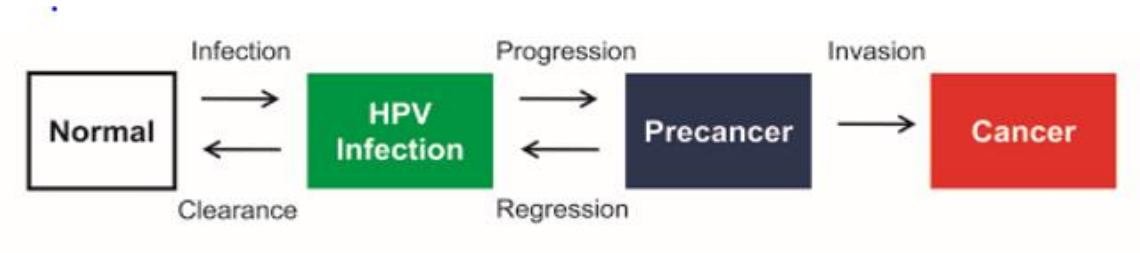


Fig.2.2.2. Natural history of Human papilloma virus. (Source Schiffman M, et al. Journal of the National cancer institute. 2011.)

Among women with persistent HPV infection, the invasive cancers occur 15-20 years later i.e. at the age of 30 years onward and peaks between 40 to 50 years. Cervical dysplasia generally is detectable up to 10 years before cancer develops, with a peak dysplasia rate, at about the age of 35 (Fig 2.2.3).

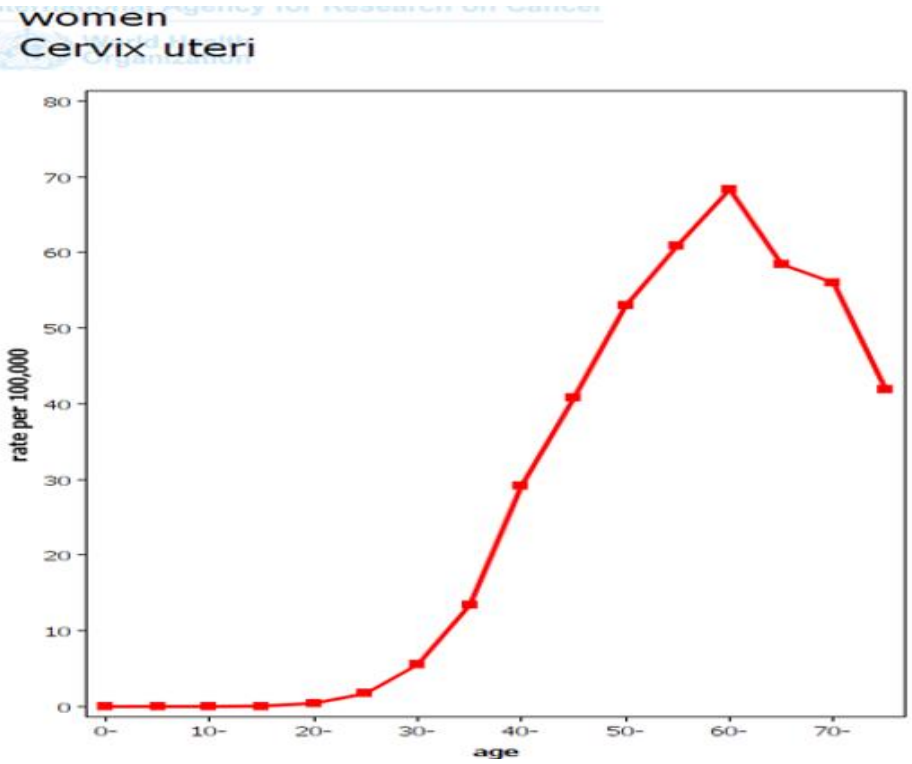


Fig.2.2.3. Age standardised incidence rate of cervical cancer among Indian women. (Source International Agency for Research on Cancer (IARC) reflecting the age for cervical cancer)

Hence as per the natural history of HPV infection, screening for cervical cancer should initially focus on women at the highest risk of cervical dysplasia i.e. women in their thirties.[12,85,87]

2.2.3. Types of Human papilloma virus as per epidemiological classification.

The International agency for Research on Cancer (IARC) Working Group uses the following classification to classify HPV types based on sufficient, limited or inadequate epidemiologic evidence for cervical carcinogenicity. IARC classifies carcinogens categorically as carcinogenic (Group 1), probably carcinogenic (Group 2a), possibly carcinogenic (Group 2b), not classifiable (Group 3), or probably not carcinogenic (Group 4). As per the reclassification of HPV types in 2009, types 16,18 remain the main cause for cervical cancer. HPV types 51,56,39,59 were added to previously known carcinogenic list (Group 1). HPV types

30,34,67,85,97,68,73,26 have not been well studied, though there is strong emerging evidence of type 68 associated with cervical carcinogenesis. The IARC working group classified type 68 to group 2a and rest to group 2b.[8,9]

Table 2.2.1. Epidemiology classification of HPV types associated with cervical cancer

High risk types	16,18,31,33,35,39,45,51,52,56,58,59,68,73,82
Low risk types	6,11,40,42,43,44,54,61,70,72,81
Intermediate types	26,53,66

At present, there are about 15 HPV types considered to be cancer-causing, or oncogenic types. The low risk, non-oncogenic HPVs are mainly responsible for cutaneous lesions (genital warts). HPV types 26,53,66, at present are classified as Intermediate types due to their rare though emerging occurrence in cervical cancer cases (Table-2.2.1).

2.2.4. Types of Human papilloma virus as per Genotypes (Fig 2.2.4)

HPV are classified into five genera designated by letters of the Greek alphabet – Alpha, Beta, Gamma, Mu, and Nu – according to the identity of the nucleotide sequences of their genomes.[9,10,88,89] The common infection caused by Human Papilloma virus are cutaneous warts which are benign in nature. These warts are caused by Gamma, Nu, Mu and cutaneous Alpha virus groups, type 2 and 52. Beta papilloma virus are second most common group of viruses reported and are harboured on skin surface and mainly remain asymptomatic.

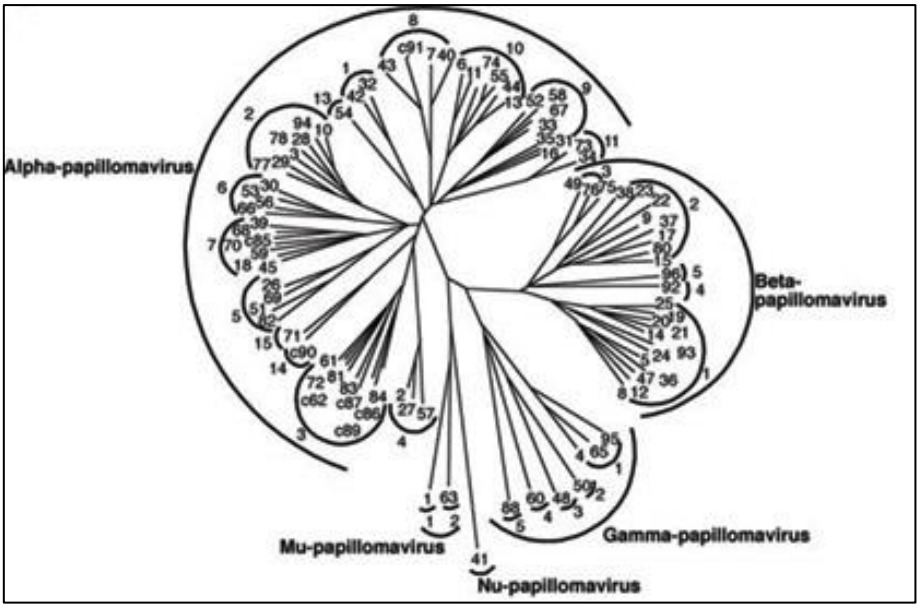


Fig 2.2.4. The Genotypes of Human papilloma virus

Among the various genotypes of HPV, the genus Alpha papillomavirus has the potential to infect both the cutaneous and mucosal epithelium in humans and cause most of the anogenital and oral mucosal infections. This genus of HPV is the largest and are of medical concern. These group of viruses are also known to cause genital and head and neck cancers. The Alpha genera of HPV are subdivided into two main branches (clades) the High risk and Low risk Alpha clades. These clades are further divided into species. The high-risk (oncogenic) Alpha type consists of species of viruses that are sexually transmitted and contribute as the main cause of cervical cancer. Low risk Alpha clade consist of species type, which cause mucosal or mucocutaneous warts that are noncancerous. The sharing of genotypes mainly of the Alpha clades which are epidemiologically classified as oncogenic and non-oncogenic viruses form the basis of cross protection to its non-vaccine genotype species by HPV vaccine[90,91] and cross reactivity of probes of Hybrid Capture 2 test, leading to false positive test results.[92,93]

2.2.5. Prevalence and common types specific Human papilloma virus

2.2.5.a- Global scenario

1. Global Prevalence of HPV

The meta-analysis conducted by Burni et al[55] to estimate the prevalence and trends of HPV during the period between January 1995 to May 2009, reports overall global age adjusted prevalence of HPV infection at any given point of time in healthy women aged between 25 to 64 years to be 11.7% (including high and low risk viruses). The prevalence of HPV also varied with the type of molecular test used. The prevalence of oncogenic viruses tested by HC2 among a subgroup of 812,677 women included in the study, the author reported overall age adjusted prevalence of HPV to be 5.7%. The highest burden of HPV was reported from Africa. Compared to the results of metanalysis reported earlier by De Sanjose et al,[94] the highest prevalence of the HPV infection reported from Africa remained the same with a comparable rates of HPV prevalence for Africa and Asian continents. The prevalence of HPV infection reported from European countries increased from 8.1% to 14.2% in the present study.[55,] The prevalence rates of HPV for Central America and Mexico along with North America showed a significant decrease in the study.[55]

Krishnakumar et al reported the prevalence of HPV to be 32.1% among 576,281 women screened from Asian countries. The highest prevalence was reported from Eastern Asia (China) followed by South Central Asia (Indian subcontinent). The author reported higher prevalence of HPV among women in the less developed regions (42.2%) as compared to women in the more developed regions (22.6%).[95]

2. Common type specific HPVs - reported globally

HPV type 16 is reported to be the commonest prevalent HPV globally irrespective of the region or countries.[55,95] HPV-18 followed by oncogenic types, such as types 52, 31, 58, 39, 56, and 51, are the next most common high-risk HPV types reported. HPV type 31 is the second commonest HPV type reported among European countries, Latin America and Caribbean while HPV type 52 is the second commonest HPV type reported from the African countries (Table 2.2.2).

Table 2.2.2. Adjusted HPV prevalence and the top two common global HPV types reported

Region	Overall Adjusted HPV Prevalence % (95%CI)	Top two HPV types
Africa	21.1 (20.2-22.0)	16 (3.5%), 52 (2.4%)
Latin America & Caribbean	16.1 (15.8-16.4)	16 (3.3 %), 18 & 31 (1.2%)
North America	4.7 (4.6-4.7)	16 (5.8%), 18 (2.3%)
Europe	14.2 (14.1-14.4)	16 (4.8%), 31 (2.3%)
Asia	9.4 (9.2-9.6)	16 (2.5%), 18 (1.4%)

Source Bruni L, et al. Journal of Infectious Diseases. 2010.

2.2.5.b. Indian scenario

1. Prevalence of HPV in India

The overall adjusted HPV prevalence rates reported for India is 7.1%.[55] Among the population-based studies the prevalence of HPV among women varied within the regions from 6.1% to 17.7% using HC2 test. Since the present study focuses on HC2 test, the search was done for studies using HC2 test assay to diagnose HPV from India (Table 2.2.3). The highest HPV

prevalence is reported from South India which also supports the highest prevalence of cervical carcinoma reported from the southern part of the country.[24,52,77,96-98]

Table 2.2.3. Cross-sectional studies showing prevalence of cervical HPV among general population of India using HC2 tests

Author	Region	Age group	Sample size	Prevalence
Franchie et al[96]	Dindigul (Tamil Nadu)	16-59	1891	17.7%
Sauvaget et al[77]	Osmanabad (Maharashtra)	30-59	27,192	10.3%
Datta et al [97]	Delhi slums	16-24	1300	8.4%
Shastri et al[24]	Mumbai (Maharashtra)	30-65	4039	7.6%
Gravitte et al[98]	Medchal Mandal (Andra Pradesh)	30-60+	2295	10.3%
Shankeranarayana et al[52]	Suburbs Kolkata	25-60	6568	6.1%

2. Common type specific HPVs – reported in India

There are limited population-based studies from India demonstrating the type specific HPV infections in general population. The studies varied with the type of PCR test used. In a study conducted at Dindigul India, the author reported HPV type 16 to be the commonest HPV type, followed by HPV 56, HPV 31, HPV 33 and HPV 18 in decreasing order using PCR probes for 44 low risk and high-risk HPVs types.[96] Similarly, the study by Datta et al in Delhi slums on 1300 married women in the age group of 16-24, reports type 16, followed by type 52 and 51 to be the commonest type specific HPV in general population. The HPV genotyping was done by using reverse blot assay which detects 37 genotypes of HPV.[99] Among the cross-sectional hospital-based study conducted in India to demonstrate the common HPV types associated with cervical cancer, type 16 and 18 were consistently reported the common HPV types followed by type 33,35,45 and 31, while type 51,39,56,58 and 59 were rarely reported to be associated with cervical cancer in India.[100-104]

2.2.6. Prevalence of Human papilloma virus by age

Globally age-related prevalence of HPV varies across the continents with some continents showing a bimodal peak in younger age and second peak during perimenopausal or postmenopausal age. The peak of HPV infection is common in younger age group with the onset of sexual activity. Some regions of Central America, South America and Africa have demonstrated prominent second peak of HPV infection 40 years onwards. A less prominent second peak of HPV infection was reported for Southern Asia by Bruni et al at around 55-64 years. The author concluded that the exact cause of this second peak remains unknow.[55] A prospective study conducted by Philip E Castle et al showed a significant increase in trends of viral prevalence in the older age group. The author reported this trend to be due to decrease in immunity to clear the HPV infection at older age rather than due to acquiring a new infection.[105] A cross-sectional population-based study that enrolled 2501 women in the age group of 25-65 years in Eastern India to study the type specific prevalence of HPV, reported higher prevalence of HPV in the age group of 20-34 years. This is expected due to natural history of HPV virus. The study did not demonstrate second peak of HPV in older women.[106] Similar findings were reported in the Dindigul study (South India) that enrolled women in age group of 16-59 years and in Mumbai study (West India) which enrolled women in age group of 30-65 years. No bimodal peak of HPV was demonstrated in all these studies.[24,96]

2.2.7. Pathophysiology of Human papilloma virus (Fig.2.2.5)

The cervix has stratified nonkeratinized glycogen containing epithelium. Histologically the epithelium of cervix consists of basal, parabasal, intermediate, superficial cell layers. The basal layer is the only layer which is mitotic and produces daughter cells which differentiate and matures to intermediate cell layer and then finally to superficial layer. Overall the cell

undergoes maturation from basal to superficial layer of epithelium, and changes occur in nucleus size, the cytoplasm content, glycogen content and the size of cell. The basal cells in the transformation zone (area between the old and the new squamo-columnar junction) of the cervix are most actively multiplying. This supports viral production. This zone is also most susceptible to HPV infection. The HPV infection enters the basal layer of the cervical epithelium by a breach in epithelium caused by trauma or inflammation.

The HPV genome consists of a circular double-stranded, 8000 bp long DNA with three regions: The upper regulatory region which functions as a transcription and replication control region, an 'early' region encoding proteins (E1, E2, E4, E5, E6, E7) for replication, regulation, modification of the host cytoplasm and nucleus and a 'late' region encoding for the viral capsid proteins (L1, L2).

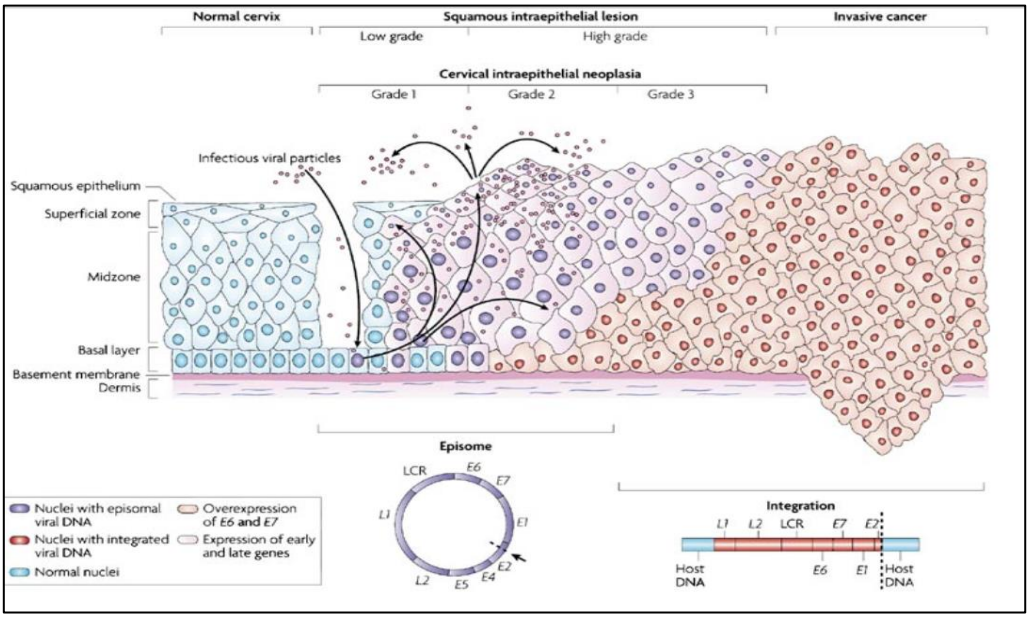


Fig.2.2.5. The pathophysiology of cervical Human papilloma virus. (Source Woodman CB et al. Nat Rev Cancer. 2007.)

After entering the basal cell layer, the early HPV genes proteins E1, E2, E4, E5, E6 and E7 are expressed and the viral DNA replicates to form episomal DNA (purple nuclei). The HPV DNA episomes are maintained as low copies in basal cells.

At this stage, the expression of E6 and E7 oncoproteins are necessary for further viral replication. In normal life cycle of the cell, regulation of E6/E7 is tightly controlled by p53 protein and pRb (retinoblastoma protein), which is a negative regulator of cell cycle, thus preventing the manifestation of the infection. In presence of any co-factors (mainly the STIs and immunity) needed for the progression of HPV infection i.e. during the productive phase of infection, the regulation of E6 and E7 is disrupted. E7 disrupts pRb from its binding to E2F, which is a transcription protein and leads to expression of some key cellular proteins like p16, MCM, PCNA necessary for viral replication. The viral genome replicates as the cell move from basal to superficial layer of cervical epithelium. E4,E1,E5,E2 are viral replication proteins that are expressed in the intermediate or superficial cell layer of epithelium during the infection phase. The E4 proteins increases the copies of viral genome, and also helps viral genome to be amplified to higher level. It encapsulates the viral genome that are then shed from surface of cervical epithelium. Mere infection of cells with HPV does not cause cancer. The initial viral replication causes some mild nuclear changes in the cell in form of vacuoles also known as koilocytosis. At this stage it may cause precancerous lesion that can be reversed. The persistent infection causes combined action of E6/E7 leading to uncontrolled cell proliferation bringing in chromosomal instability and the virus finally gets integrated leading to overexpression of E6/E7 aiding lesion(s) to progress from precancerous to cancerous form(s) (red nuclei).[107,108]

2.2.8. Incubation period and route of transmission of Human papilloma Virus

HPV is infectious, with incubation periods ranging from 2–4 weeks to months or years. The duration of this phase of latency probably relates to the dose of virus and the cell mediated immunity of an individual.[89] John Doorbar et al demonstrated that the time taken for an infected basal cell to be exfoliated from epithelial surface to be around 2-4 weeks.[107] Winner et al in his

prospective study demonstrated latency period of cervical HPV to be 20 days and skin to skin transmission to be a recognised mode of HPV transmission other than penetrative sex.[109]

2.2.9. Co-factors for persistence of Human papilloma virus

It is a known fact that HPV infection is causative factor for cervical cancer. It is also known that 80% of the women are infected with HPV infection atleast once in their lifetime. In most cases HPV infections clear spontaneously. Presence of co-factors contribute to persistent HPV infections, making women prone to cervical cancer. The recognised co-factors are use of Oral contraceptives (OCs), tobacco smoking or tobacco chewing, cervical trauma related to parity, coinfection with human immunodeficiency virus (HIV) and other sexually transmitted agents.

1. Role of Parity as a co-factor

The studies before year 2000 demonstrated higher parity and lower interval between pregnancies as causative factors for cervical cancer, while abortions showed no influence on incidence of cervical carcinoma.[110,111] The hypothesis stated was early age of marriage and repeated childbirths are known to be associated with more wear and tear of cervical tissue, thereby increasing the risk of cervical cancers caused due to trauma. The major limitations of these studies were failure to account for the causative role of HPV, which is now recognized cause of cervical cancer. The pooled analysis of case control studies carried out by Nubia Munoz et al in 2002 and Salvatore et al in 2006 taking into account parity as a co-factor to increase the risk of cervical cancer in HPV infected women, demonstrated conflicting result.[112,113]

The emerging evidence from prospective studies demonstrates parity to be modulating the progression of HPV infection to cervical cancer.[15,16] Pregnancy related hormones cause cervical ectropion causing transformation zone to lie on ectocervix. The actively dividing cells

at the transformation zone makes women prone for acquisition and persistence of high-risk HPV. This is a pre-requisite for HPV infection to progress to cervical cancer. Moreover, the trauma caused to cervix during delivery can also contribute for acquisition and persistence of HPV. These factors explain role of parity as a co-factor in the development of cervical cancer.

2. Role of Oral contraceptive (OCP) use as a co-factor

There is a renewed concern about the possible relationship between long term use of OCP and cervical cancer. The pooled analysis of case control studies that assessed the role of oral contraception as a co-factor in increasing the risk of cervical cancer among women infected with HPV, demonstrated increased risk of developing cancer with increased duration of oral contraceptive (OCP) use, after 5 years. The limitations of the above study were selection and recall biases in terms of accuracy in recalling the use of pills by the women. The above study was a cross-sectional study which took into account only transient HPV infections and we understand the persistence of HPV infection is necessary to cause cervical cancer.[114] The other prospective longitudinal studies which looked into the role of OCP in persistence of HPV failed to demonstrate the association between OCP and cervical cancer.[115] With respect to cervical cancer, the benefits of OCP outweigh the risk, because the number of cervical cancers resulting from the use of OCP is very small and therefore women should not be discouraged from using the pills. The association between OCP use and cervical cancer can be complicated by possible confounding factor of sexual behaviour. Further prospective studies are warrant to demonstrate OCP in persistence of HPV infection.[15]

3. Role of Tobacco as a co-factor

Currently smoking among women remains an independent predictor for cervical carcinogenesis. Tobacco can itself be a risk factor for the development of cervical cancer due to the ability of carcinogens in cigarette smoke to cause mutations in DNA of cervical cells due to the nicotine content. The determinants of smoking can be unhealthy lifestyle, lack of social support, stress, distress etc which can make women prone for high risk behaviour, an important factor for sexually transmitted diseases including HPV infection. Smokers have a low local and/or systemic cell mediated immunity and seem to have lower compliance for cervical cancer screening, that makes them prone for cervical cancer.[116] Smoking affects the clearance of HPV and helps in progression of HPV infection to precancerous or cancerous lesion.[15,115,117]

4. Low socio-economic status and STIs.

Socio-economic status has an important correlation with cancer or STIs (sexually transmitted disease). The determinants of low economic status are mainly the low education, low income and poverty status that varies across countries. Women who are less educated may not be aware of the symptoms pertaining to precancer and cancer, and hence usually present at advanced stages of cancer. Moreover, they have restricted access to health care facilities for screening and treatment. Nutritional deficiency, poor genital hygiene, limited or no contraceptive knowledge make them vulnerable to repeated pregnancies and recurrent STI infections, which is one of the co-factors for HPV infection. It is postulated that individuals with low income or below the poverty line are more likely to engage in high risk sexual activity making them prone for STI/RTIs including HPV. The STI/RTIs play a major role in persistence and progression of HPV infection to precancerous and cancerous lesions of cervix.[13,14,118,119.]

5. HIV and HPV

Women infected with HIV are prone to be infected with high-risk HPV types and have increased chances of persistent HPV due to decreased cellular immunity. They are more likely to develop precancerous lesions (development is rapid) than HIV-negative women in the same age group.[120] It is known that, lower the CD4 counts, higher the risk of HPV acquisition from infected sexual partner.[121] The seropositive women are also prone to harbor multiple types of HPV.[122] A study done by Howard et al showed that in sexually active HIV-positive women, plasma HIV RNA level and $CD4^{+}$ count in combination appeared to have a strong and statistically significant association with incident detection of HPV and HPV reactivation due to low cellular immunity.[18]

2.3 Burden of Reproductive tract infection and its role as a co-factor for persistent Human Papillomavirus infection.

2.3.1. Burden of Reproductive tract infections globally

Worldwide the major cause of STI/RTIs leading to cervical inflammation or cervicovaginitis reported are Gonorrhoea, Chlamydia, Trichomoniasis, candidiasis, Bacterial Vaginosis and Mycoplasma genitalium.[56] World Health Organization (WHO) states that more than one million STIs are acquired everyday, worldwide. Each year, WHO estimates 357 million new infections with one of the four bacterial STIs: Chlamydia, Gonorrhoea, Syphilis and Trichomoniasis globally (Fig 2.3.1). There is increasing trends of viral STIs reported, mainly Hepatitis B, Human immunodeficiency virus (HIV), Herpes simplex virus (HSV) and Human papilloma virus (HPV). Majority of STIs among women are asymptomatic and are known to cause serious reproductive health issues. WHO reports, major burden of STIs to be contributed by developing countries.[58]

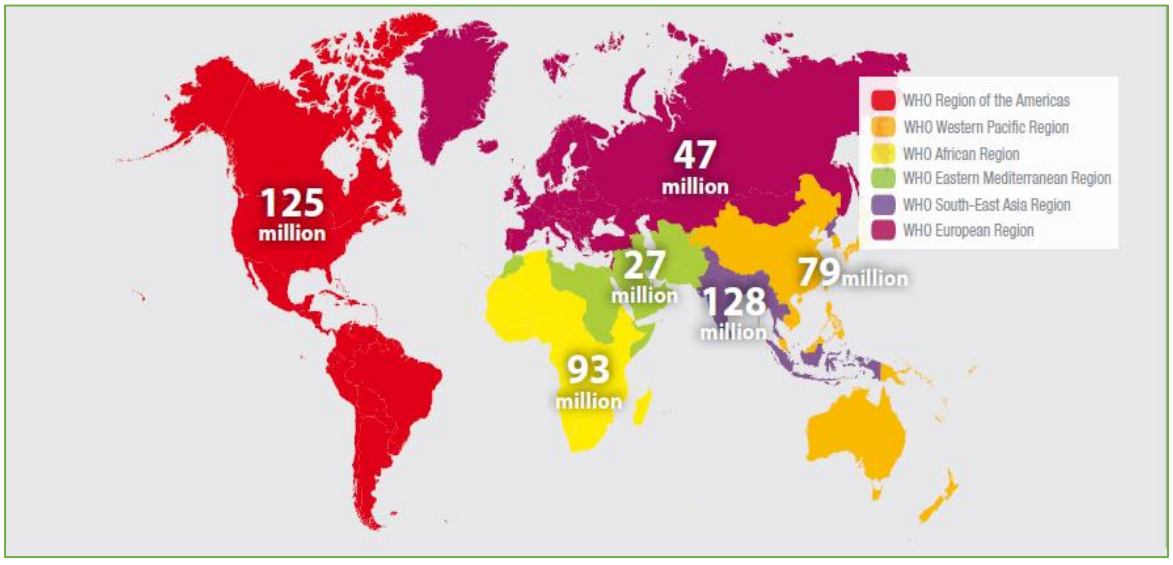


Fig 2.3.1. Burden of Sexually transmitted infections reported worldwide. (Source-WHO regionwide estimated new cases of Gonorrhoea, Chlamydia, Syphilis and Trichomoniasis. 2008)

2.3.2. Burden of Reproductive tract infections in developing countries

High income countries have established quality screening and diagnostic tools for RTIs. The burden of RTIs are expected to be high among middle- and low-income countries, as these countries lack development of quality care and treatment for STIs/RTIs services due to resource constrains.[58]

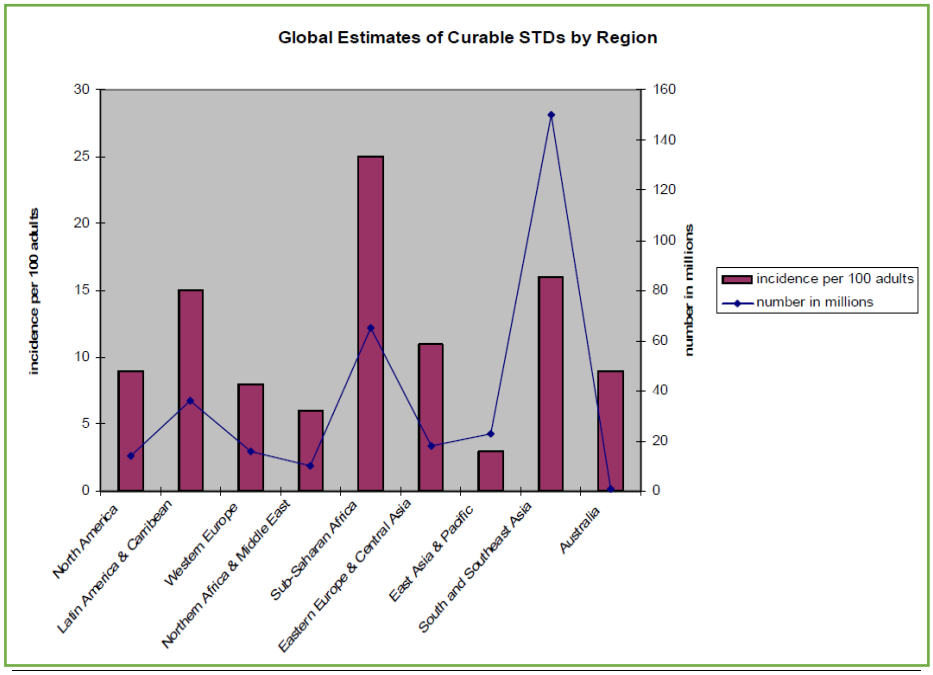


Fig 2.3.2. Prevalence of sexually transmitted infections among selected regions. (Source- CDC 2008: STI in developing countries)

The highest number of STIs are reported from Africa and Southeast Asian countries (Fig-2.3.2). RTIs are known to have a major impact on women's health and economy of the country, in terms of catastrophic out of pocket health expenditure. It becomes important to treat these RTIs due to devastating consequences on women's health, increasing maternal morbidity (infertility, bad obstetric history, Pelvic inflammatory disease, post abortal and puerperal sepsis) and mortality. The high burden of the diseases from developing countries is due to a) poor health seeking behaviour for STI/RTI treatment because of stigma associated with the disease. b) lack of point of care diagnosis for common STI/RTIs. c) absence of

effective counselling services to ensure partner treatment and self-treatment.[58]

2.3.3. Burden of Reproductive tract infections in India

Inspite of several policies and programmes run by the Indian government like National Rural Health Mission and Reproductive & Child Health program that have a component of RTIs screening and treatment, the burden of disease still remains high in India. This is due to illiteracy, ignorance, social stigma, cultural norms, lack of quality health care facilities for screening and treating RTIs and poor access to health care facilities, if available.[58,59,123]

The accurate data regarding the prevalence of STI/RTIs is lacking from India. The point prevalence of STIs/RTIs in India is estimated to be around 30 million. Among these 50% of women are reported to be asymptomatic.[56,59,124]

A marked heterogeneity is seen in the prevalence of STI/RTIs among women in the general population in India. It varies across different states in India (Table 2.3.1). The cause of these variations in prevalence rates across regions can be attributed to the level of awareness pertaining to RTIs screening, laboratory methods used for diagnosis and interpretation of results.[59,63,72,125-133] In various cross-sectional community based and hospital-based studies conducted in India, the most common RTIs reported are Bacterial Vaginosis (BV) followed by Candidiasis. Among the bacterial STIs, the overall prevalence of Neisseria gonorrhoea and Chlamydial infections is comparatively reported less. Ray et al in their study reported the prevalence of Gonorrhoea and Chlamydia to be 1.1% and 2.4% respectively in symptomatic women and 0.4% and 1% respectively in asymptomatic women.[59] The similar findings of endogenous RTIs infections (Bacterial vaginosis, Candidiasis) being more frequent than STIs is also supported by a large population based cross-sectional trial conducted by Patel et al from India.[63]

Table 2.3.1. Cross-sectional studies from India demonstrating the most common bacterial Reproductive tract infections among women

Authors	Location	Sample size	1st common RTI	2nd common RTI	Overall Lab RTI Prevalence
Population based studies					
Parmar et al[72]	Thane Mumbai	415	Candida (46.3%)	BV (25%)	26%
Bote et al[125]	Mumbai urban slum	466	Candida (37%)	BV (13.9%)	67.81%
Prabha et al[126]	Medak AP	407	BV (14.3%)	Candida (6%)	33%
Patel et al[63]	Goa	2494	BV (17.8%)	Candida (8.5%)	-
Balamurugan et al [127]	Hubli Karnataka	658	Candida (16%)	BV (12.5%)	40.4%
Jindal et al[128]	Rural Punjab	200	BV (11%)	Candida (45%)	20%
Rao et al[129]	Tribal Jabalpur	2206	BV (3.1%)	Tricho (1.9%)	6.4%
Hospital based studies					
Ray et al [59]	Delhi PHC	1105	Candida (22.1%)	BV (5.5%)	27.6%
Chauhan et al[130]	Gynaec Dept. Gujarat	180	BV (26.1%)	Candida (8.3%)	42%
Vishwanath et al[131]	Gynaec Dept Delhi	319	BV (26%)	Candida (26%)	76%
Kaur et al.[132]	Gynaec Dept. Patiala	500	BV (57.5%)	Candida (45.86%)	58%
Ray et al[133]	Delhi NACO clinic	2035	Candida (28.5%)	BV (4.5%)	30%

Bacterial vaginosis- Range 3.1% -57.5% **Candida- Range 6%-45.86%**

Similar findings were reported by the mid-term review report of National AIDS Control program (NACO) India, Phase three (NACP III) survey conducted in Nov-Dec 2009. The report mentions a considerable change in the trends of bacterial STI/RTIs in India. There is a considerable increase in burden of RTIs (Candidiasis/ BV) than STIs (Gonorrhoea/

Chlamydial infection) reported among the Indian female population. The prevalence of candidiasis and BV among women was between 7.2% to 23.9% and 17.8% to 63.7% respectively. The prevalence of Gonorrhea was ≤ 1.9%, and Chlamydial infection ≤ 1.3%. [61] These figures of prevalence of Gonorrhoea and chlamydia reported may be underestimated due to lack of appropriate cost-effective screening tool in resource constrained country like India for these organisms.

A large hospital based study conducted among 4090 women attending peripheral health centre in Delhi to establish the prevalence of STIs/RTIs among four groups of women -symptomatic women, women with bad obstetric history, pregnant women and asymptomatic women, reported an overall prevalence of RTIs (Bacterial Vaginosis and Candidiasis) to be 24.3% and the prevalence rates of STIs (Gonococcal, Chlamydia, Trichomonas, Hepatitis B, Herpes simplex-2, HIV, Chancroid) to be 12.5%. The prevalence rates varied among different categories of women. The author reported the prevalence rate of RTIs to be 15.2% and STIs to be 9.9% in asymptomatic women.[59]

The most common age group for STI/RTIs reported among Indian women is 25-44 years.[57]

The burden of STIs is higher in men than women. Therefore, the strategy of treating male partner remains the hardcore of STI management.[134] There is also an emerging evidence of increase in viral STIs reported from India.[62]

Among the viral STIs there is an increasing trends of Human Immunodeficiency Virus (HIV) which causes acquired immunodeficiency syndrome, Herpes Simplex Virus-2 (HSV-2) associated with genital herpes and Human Papilloma Virus (HPV) that constitutes a major cause of genital warts and genital cancers in both genders. Among the viral STIs, HPV infection remains a major concern for its association with cervical cancer. Cervical cancer is reported to be the fourth most common cancer in women globally and is the second most common cancer among Indian women.[1] Among the HPV types reported-low risk HPV and

high-risk HPV (oncogenic HPV), there is a strong causal association between persistent genital tract infection with oncogenic HPV and cervical cancer.[7,10]

2.3.4. Co-infection of Reproductive tract infections and Human Papilloma virus

The bacterial STI/RTIs causes inflammation of cervix (cervicitis) leading to unhealthy cervix that bleeds on touch due to break in cervical epithelium and is associated with purulent or mucopurulent discharge. The change in vaginal microbiological flora and inflammation associated with cervicitis may help in acquiring and transmitting HPV infection which is the main cause for cervical cancer. The break in cervical epithelium helps HPV to gain entry in actively proliferating basal cell layer of epithelial which is a primary step in natural history for cervical HPV infection.[13] Certain inflammatory mediator like reactive oxygen and nitrogen species (ROS and RNS) that are produced due to cervical inflammation, are proposed for causing damage to host cell DNA leading to integration of viral genome into the host cell genome. This serves as the main step for oncogenic HPV infection to gradually progress to cervical cancer.[14]

There are limited studies reported from India demonstrating the burden of clinical cervicitis in general population. Parmar et al reported 55.2% prevalence of clinical cervicitis in a community-based study conducted in rural Thane among 415 women in reproductive age group.[72] Kaur et al reported 11.6% prevalence of cervical erosion among women with a history of vaginal discharge in a hospital-based study.[132]

This difference in prevalence of cervicitis may be due to poor hospital seeking behavior among Indian women. The women with less formal education are less aware of initial signs and symptoms of RTIs and is less likely to be aware of screening facilities in the nearby area. The women usually present late in the hospital when the complications set in.

It is important to screen and treat women with cervicitis promptly, as cervicitis caused by STI/RTIs acts as co-factor for persistent oncogenic HPV, which is the main cause of cervical cancer. Studies conducted in China, Costa-Rica, Brazil, Turkey to estimate the prevalence of oncogenic HPV among women with cervicitis/ Mucopurulent cervicitis reported the prevalence of the infection to be 5%-64%.[64-66,135,136] These studies varied in the study design and the method of diagnosing cervicitis. To our knowledge there is no study from India till date documenting the prevalence of HPV among women with cervicitis (Table- 2.3.2).

Table 2.3.2. Prevalence of HPV among women with cervicitis demonstrated using molecular HPVDNA test

Author/year	Country	Study design	Sample size	Diagnosis of cervicitis	Prevalence of HPV in cervicitis
Liuw WEQ et al (2013)[66]	China	Case-control	191	Clinical	53%
Philip E Castle et al (2001)[64]	Costa-Rica	Nested case-control	445	Cervicitis on Pap smear	58.7%
Rodrigo Cesar et al (2015)[135]	Brazil	Cross-sectional	251	Cervicitis on Pap smear	44%
Yuan X et al (2011)[65]	China	Cross-sectional	4601	Clinical	11%
Altuglu et al (2002)[136]	Turkey	Cross-sectional	148	Clinical	5%

There is also an emerging evidence of concomitant genital STIs and HPV infection of the uterine cervix. There is a strong emerging evidence for an association of BV and uterine cervical oncogenic HPV infection.[68-71,137] The similar association has also been reported for Chlamydia and cervical oncogenic HPV.[119] A case control study conducted by Smith JS et al reported the risk of squamous cell carcinoma with co-infection with Chlamydial infection to increase by 2.1 odds (CI- 1.1–4.0). The author reported no significant risk for adenocarcinoma of cervix associated with Chlamydial infection.[68] At present, among the other STI/RTIs, the

association of persistent HPV with trichomonas infection and Candida is controversial and needs further studies.[138-141]

There is limited evidence of co-infection of HPV and RTIs among Indian women. The study in eastern India conducted to know the burden of co-infection of STIs and HPV in 45 Commercial Sex Workers, reported the prevalence of Candida, Trichomonas and Chlamydia infection in HPV positive women to be 88.6%, 22.9% and 14.3% respectively. Among the Viral STIs, HSV2 was reported to be highest with prevalence of 34.3%. The major drawback of the study was small sample size.[142]

2.3.5. Effect of Reproductive tract infections on cervical cancer screening tests

There has been a concern about blood and mucous associated with cervical inflammation caused by STIs/RTIs, interfering with the sensitivity of cytology-based screening test for cervical cancer. The multicentric trial conducted in India, that evaluated accuracy of conventional cytology, to detect CIN2+ lesions, reported the sensitivity of cytology to be substantially low among women in age group of 25–39 years.[73] Similar findings were also reported from a study from UK which demonstrated modest effect of cytology based screening test in the age of 20-39 years in preventing cervical cancer.[74] The probable reasons stated by the author for low sensitivity of cytology in the above age group are[73] a) Presumably high frequency of RTIs among young women that may interfere with cervical samplings and reading of smears. b) Many Indian women have less formal education and hence may be more prone for RTIs due to poor genital hygiene. c) In the above age group there lies a possibility of interference with blood, because of the presence of large transformation zone resulting from ectropion. These factors may account for low sensitivity of cytology, due to interference of blood and mucous associated with inflammation of cervix or ectropion. The cervical inflammation has a potential to affect the interpretation of Visual Inspection of acetic acid

(VIA) test results. There are rapid developments in molecular screening tests for cervical cancer screening. The HPV molecular screening test has an advantage, as it detects women with HPV, the primary cause for cervical cancer. Among the molecular test the HC2 test and Polymerase chain reaction (PCR) has been evaluated in various settings worldwide.[143,144]

Kuhn et al[50] based on his study to evaluate the test characteristics of HPV DNA HC2 as a screening test among African women, states that HPV DNA test is unlikely to be adversely influenced by cervical inflammation due to STIs, which are common conditions among women in low resource settings. The main limitation of the study was that the study was not powered to demonstrate the effect of STIs on test characteristics of HC2 test.[50] A study conducted among Chinese women to estimate the prevalence of HPV genotype among three groups of women; women with mucopurulent cervicitis, healthy women and women with Invasive cancer using PCR (GP5+/GP6+ and PGMY09/11 L1 consensus primers). The women tested positive with HPVDNA, were subjected to sequencing for HPV genotyping. Amplification of β-globin DNA was performed as a surrogate marker to test the sample adequacy. The author reported 10% higher failure rates to extract HPV DNA in cases of mucopurulent cervicitis as compared to the other two groups due to degradation of HPVDNA.[66] This study shows the potential of HPVDNA test to be influenced by RTIs.

2.4. Hybrid Capture 2 as a screening test in developed countries and it's issue in developing countries

2.4.1. Advantages of molecular based HPVDNA screening for cervical cancer

There is sufficient evidence from epidemiological studies about the causal role of persistent oncogenic Human papillomavirus (HPV) infection as a main cause for cervical cancer.[7,10] The various screening tests aid in the diagnosis of precancerous stage of cervical cancer, a stage where women are totally asymptomatic and treatment given has a high chance of cure. The cytology-based screening has limitation in terms of cell inadequacy during sample collection (improper collection), transportation issues and interpretation skills of laboratory personnel. Cytology-based screening necessitates short screening intervals due to its low sensitivity.[29] This contributes to logistic challenges, especially in developing countries, in terms of follow up. The direct detection of HPV in cervical specimen by a molecular test can offer an alternative for cytology-based screening.

Among the various molecular tests approved by the Food and Drug Administration (FDA), the HPVDNA Hybrid Capture 2 test (HC2, Qiagen, Gaithersburg, MD) has been extensively evaluated.[143,144] The HC2 HPVDNA molecular test identifies women at risk of developing cervical cancer, needing a close follow up. The test has probes for 13 oncogenic HPV types, commonly reported worldwide.[55]

The advantages of HPVDNA molecular based screening test[29,143] over cytology-based screening are- a) high sensitivity reported from developing countries. b) longer screening interval if tested negative. c) requirement of minimal resources and manpower for performing the test. d) - the potential of self-collection. e) the reproducibility and standardization of the test with no inter and intra subjective variability.

2.4.2. HVPDNA HC2 test in developed countries.

The population based cross sectional studies from Switzerland, France, UK (HART study), Japan, Great Manchester (ARTISTIC trial), Germany (Montreal & St John's church) and Italy evaluated HC2 test versus conventional or liquid based cytology to investigate the best screening modality for cervical cancer.[39-47] The second generation HC2 test assay was used. The Relative light unit (RLU) titers of 1/> 1 RLU corresponding to 5000 or more copies of the virus per ml was interpreted as a positive test.

The above cross-sectional studies from the developed countries, focused to determine the sensitivity and specificity of HPV HC2 to determine CIN2+ lesion demonstrated the sensitivity in the range of 90% to 100%, with an average of 95%. The specificity demonstrated is in range of 85%-95%, with an average of 91.5% (table-2.4.1). Overall HC2 test demonstrated a greater sensitivity to diagnose CIN2+ lesions than the cervical cytology as a stand-alone test.

The developed countries like Europe, United Kingdom, France, Germany, Netherland, United states and Canada have good quality cytology based cervical cancer screening program. A study involving around 60,000 women in these countries who received HPVDNA (HC2 or PCR GP5/6+ primer) as a parallel test in addition to Pap cytology in the age group 15 -80 years, reported overall sensitivity and specificity of HC2 test to diagnose CIN2+ lesions as 96.1% and 90.7% respectively as compared to Pap cytology which reported a low sensitivity of 53% and specificity of 96.3%. The study also demonstrated, the age group of 35-49 years where the likelihood of persistent HPV infections are common, the sensitivity of HC2 test reported was 93.9% versus 55.4% of Pap cytology. The sensitivity of HC2 test increased from 93.9% to 97.5% versus 55.4% to 79.3% for Pap cytology in the age group of 50+ years. After controlling for verification biases, the study supported HC2 as a primary screening test with Pap cytology as a triage test for HPV positive women.[145]

Table 2.4.1. Sensitivity and specificity of HC2 test to diagnose CIN2+ lesion demonstrated in cross-sectional studies from developed countries (year 1999-2004)[39-47]

Country	Year	Population	Age of population	Sensitivity (95% CI)	Specificity (95% CI)
Japan[39]	2003-2004	8156	14-83 years	90%	Not reported
UK (HART Study)[40]	1998-2001	11085	30-60 years	97% (91.2-99.1)	93.3% (92.7-93.9)
Germany[41]	1998-2000	8466	30 and above	97.8% (86.3-99.7)	95.3% (93.5-96.6)
Canada[42] (CCCaST trial)	2002-2004	10,154	30-69	94.6% (84.2-100)	94.1% (93.4-94.8)
Swiss[43]	2002-2004	10,154	30-69	94.6% (84.2-100)	94.1% (93.4-94.8)
Riems[44]	1997-2001	7932	15-76 years	100% (93.8-100)	87.3% (85.9-88.7)
France[45] (Public n Private)	1999-2000	2588	15-80 years	96% (88-100)	85% (83-87)
Italy (NTCC STUDY)[46]	2000-2003	33,364	35-60 years	97.3% (90.7-99.7)	93.2% (92.8-93.6)
UK[47] (Great Manchester)	2001-2003	24,510	20-64 years	93.40%	Not reported

Average Sensitivity-95% (range 90-100%) Average specificity -91.5% (range 85%-95%)

The HC2 test has been extensively evaluated to be used in various combinations for cervical cancer screening across the world. There is a strong evidence for the use of HC2 as a triage test for ASCUS cytology (Atypical squamous cells of undetermined significance) rather than repeating a Pap cytology. Using HPVDNA test as primary screening test, is reported to detect 23% more CIN2+ lesion as compared to Pap cytology alone.[35]

Huang et al monitored a cohort of 1708 healthy Taiwan women, aged 20–90 who had received a baseline Pap cytology and HPVDNA test (HC2) in May 2000. The enrolled subjects were left undisturbed and the end point data of cervical neoplasia were collected through the national database of Pap smear and cancer registry in Aug 2006. Records of 108 cytology-negative &

HPV-positive women and 1202 cytology & HPV negative women were retrospectively analyzed. The study demonstrated that women who had prevalent infection with high risk HPV had 4% cumulative risk for cervical cancer in 6 years as compared to women tested negative for high risk HPV at baseline. The author supports, HPV DNA as a primary screening test for cervical cancer with a screening free interval of atleast 5 years, if tested negative[146]. The similar finding demonstrating that longer screening interval would suffice if HPVDNA test is used as a primary screening test as compared to cytology was also supported by a recent Sweden Randomized Control Trial (RCT).[147]

There is sufficient evidence from developed countries which demonstrates HPVDNA test to have better sensitivity than cytology to detect CIN2+ lesions. The above literature supports HPVDNA test as a potential primary screening tool for cervical cancer screening where in longer screening intervals would suffice. The biggest benefits of primary screening with HPVDNA test in women is demonstrated for the age group of 35 and above**.** The current recommendation of screening guidelines for countries with good cytology based cervical cancer screening program would be to move towards HPVDNA as primary testing modality in order to safely increase the screening intervals.[148] United States has already approved HC2 as a co-testing modality for cervical cancer screening in women older than 30 years of age. European countries are on the verge of introducing the HPVDNA test as a stand-alone test for primary cervical cancer screening.[36] Australia and the Netherlands have now adopted HPV based cervical cancer screening in their national screening programs.[37,38]

2.4.3. Issue of HVPDNA HC2 test in developing countries

The largest population based cross-sectional study from rural district of Osmanabad in Western India, randomized around 131,746 women in the age group 30-59 years into four groups to undergo different cervical cancer screening modalities. First group received HPV screening by HC2 conducted by technicians, second group received conventional Pap-smear screening by cytotechnologists, third group received VIA (Visual inspection with acetic acid) screening by primary health care workers and the fourth group received standard care (no-screening). The study reported a significant 48% decrease in cervical cancer mortality after 7 years among women who had received a single round of HPV screening when compared with unscreened women. In the same study, the VIA screening and the Pap-smear screening groups did not show a significant decrease in cervical cancer mortality benefit when compared with no screening group. The study also supported that in developing countries where follow up of women is a challenge, the best option for the country would be a single round of HPV-DNA testing around the age of 35 yrs.[31]

Mexico introduced the Pap-smear screening in 1974. However, cervical cancer mortality rates remained unchanged for the country even after 15 years.[21] A study that was conducted later in Morelos (Mexico) on 7872 women in the age group of 20-80 years to evaluate the Pap cytology versus HPVDNA HC2 test as primary screening test to diagnose CIN2+ lesion, demonstrated higher sensitivity of 93.1% for HC2 test as compared to 59.4% for Pap cytology.[33] The country later introduced primary screening by HC2 test for women aged 35–64 years and HPV vaccination for girls aged 9–12 years in their national immunization program in 2008.[34]

The findings of the above studies are very encouraging as it demonstrates that a simple and reliable HPV DNA test which is now available in low income countries has a potential to be accepted as primary screening tool in the near future. The World Health Organization (WHO) guidelines has recommended molecular HPV based screening as the test of first choice in the

countries contemplating to initiate cervical cancer screening program, provided it is logistically feasible and affordable.[29] The benefits of HPV test in terms of providing single round of screening at age 35 and above, would be in terms of preventing the repeated rounds of testing that are needed for Pap cytology and VIA test.[31]

The population based cross sectional studies from developing countries like Peru, Latin America, Zimbabwe, South Africa, India, Mexico and Costa-Rica also evaluated HC2 test versus other cervical cancer screening modalities (Pap cytology/ VIA) to investigate the best screening modality for their countries. These countries also used second generation HC2 assay and the value of 1/> 1 RLU corresponding to 5000 or more copies of the virus per ml was interpreted as a positive test.[32,48-53]

The sensitivity of HC2 test assay to diagnose CIN2+ lesions demonstrated is in the range of 50% to 91% with an average of 79%. The specificity demonstrated is in rage of 62%-94.6%, with an average of 86% (Table- 2.4.2).

There is a statistically significant difference (p value = 0.003) in sensitivity of HPV DNA HC2 test to determine CIN2+ lesions between the developed and developing countries, while there is no difference in the specificity of the test. Similar findings are supported by Arbyn M et al.[149] There is an overall issue of low sensitivity of HC2 test to diagnose CIN2+ lesions reported from developing countries especially from Indian context (Table-2.4.3).

Table 2.4.2. Sensitivity and specificity of HC2 test to diagnose CIN2+ lesion demonstrated in cross-sectional studies from developing countries (year 1993-2003)[32,48-53]

Country	Year	Population	Age of population	Sensitivity (95%CI)	Specificity (95% CI)
Peru[48]	2001	5435	25-49 years	77.27% (70.4-83.5)	89.32 (88.5-90.1)
Latin America [51]	2002-2003	4195	18-60 years	82.8% (76.3-88.4)	86.4% (85.3-87.5)
Zimbabwe [49]	1996-1997	2140	25-55 years	81% (75-86%)	62% (59-64%)
South Africa [50]	1998-1999	424	35-65 years	88.4% (76.9-81.9)	81.9% (76.5-86.5)
India (K1) [52]	1999-2003	4063	25-65 years	50% (36.6-63.4)	91.7% (90.7-92.6)
India (M) [52]	1999-2003	3551	25-65 years	70.5% (57.4-81.5)	93.6% (92.7-94.4)
India (T2) [52]	1999-2003	4761	25-65 years	80% (67.7-89.2)	94.6% (93.9-95.3)
Mexico [32]	1999	7868	15-85 years	90.7% (83.4-95%)	93.2% (92.1-93.3)
Costa-Rica [53]	1993-1994	1119	18 n Above	88.40%	89.40%

Sensitivity - 79% (range 50%-91%) specificity - 86% (range 62%-94.6%)

Table 2.4.3. Test characteristics of HC2 for diagnosing CIN-2+ lesions reported from developed and developing countries.

Groups	Developed countries	Developing countries	P value
Sensitivity (average)	95%	79%	0.003
Specificity (average)	91.5%	86.9%	0.276

A cross-sectional population based multicentric study, enrolling 18,085 women in age group of 25-65 years was conducted across three locations in India to evaluate the test characteristics of HPV DNA HC2 in detecting CIN-2+ lesions. The author reported a large range of sensitivity of HC2 test varying from 54.7% to 80.9% and specificity varying from 91.7% to 94.6%. Low

sensitivity of HC2 test was demonstrated at Mumbai and Kolkata sites as compared to Trivandrum site. The author attributed the low sensitivity of HC2 test to sampling technique, leading to cell inadequacy, as the specimen was collected by nonmedical staff at Mumbai and Kolkata site and also due to differences in the sequence of test performed as HPVDNA was performed after the Pap test.[52]

Arbyn M et al in their study, evaluated the performance of five cervical cancer screening test (VIA, VIAM, VILLI, HC2, Pap cytology) from Sub-Saharan African countries and India to diagnose CIN2+ lesions. The author reported a pooled sensitivity of 62% (95% CI 56–68%) and a specificity of 94% (95% CI 92–95%) for HC2 test for diagnosing CIN2+ lesion from these countries. The sensitivity of HC2 test for diagnosing CIN2+ lesion was reported to be lower than that of VIA (though not statistically significant). The author attributed this low sensitivity reported from the developing countries to deterioration/ degradation of the HPVDNA sample due to exposure of DNA to high temperature during transportation, misclassification of the outcome depending on gold standard (either colposcopy or biopsy) and prevalence of HPV type not included in B probe cocktail of HC2 test.[149] The similar cause of degradation of HPVDNA in the sample due to improper temperature during transportation and storage was also reported by Almonte et al. Their study reported low sensitivity of HPVDNA HC2 test for CIN2+ lesions, around 77.27% and the specificity of 89.32%. The study was conducted in Peru, wherein the health care facility was difficult to access due to poor road quality. The collected HPVDNA samples were sent to London for HC2 testing. The study also reported huge loss to follow up of referrals cases.[48]

Among the cross-sectional study conducted in Harare, Zimbabwe[49] and Latin America[51] the authors demonstrated moderate sensitivity of HC2 test (81% and 82.8% respectively) for CIN2+ lesion. The authors proposed the HC2 RLU cutoff assays and the prevalence of HPV in a particular geographical area to affect the sensitivity of the test assay.

The study from South Africa,[50] primarily evaluated HPVDNA HC1 test at various RLU cut off >/= 1 as an alternative screening test for Pap cytology in low resource setting. A subset of stored HPVDNA samples of women with biopsy confirmed precancerous and cancerous lesions and a 10% random sample of women with no cervical disease were retrospectively retested using HC2 test. The author reported 88.4% sensitivity of HC2 test to diagnose CIN2+ lesions which is comparable with the sensitivity reported from developed countries. The author attributed the high sensitivity of HC2 test to high prevalence rate of HPV (22%), HIV (8%) and STI (6% Gonorrhoea /Chlamydial infection, 18% Trichomonas infection) burden among women enrolled in the study at subsequent visit. The limitations of the study were verification biases, small retrospective sample and high risk of the population for HPV. The findings of this study cannot be generalized. The other study from developing country with a comparable sensitivity of HC2 test (90.7%) with other developed countries was reported by Salmeron et al[32] after adjusting verification biases due to factors like loss to follow up, unconfirmed cases due to sample inadequacy.

The cross-sectional population-based study conducted in Mumbai[24], as part of multicentric trial[52] to evaluate the sensitivity of HC2 test to diagnose CIN2+ lesion, reported 62% sensitivity for detecting CIN2+ lesions after controlling the known factors affecting the test sensitivity like- the sample collection technique and the transportation of HC2 from field settings to laboratory as per the SOP of Microbiology Dept. The staff underwent regular training on sample collection technique and had regular rounds of quality control. There was no verification bias, as all women enrolled in the study underwent colposcopy and biopsy was taken for cervical abnormalities. The samples of HC2 were processed at Microbiology department which has a NABL accreditation.

2.5. Laboratory tests for common Reproductive tract infections and Human Papillomavirus infection

2.5.1. Laboratory diagnosis of Reproductive tract infections

The vaginal microbiological flora of the lower female genital tract is a complex of aerobes and anaerobes bacteria which are under the influence of factors like age, oestrogen levels and lactobacilli. The infection of lower genital tract infections are caused by exogenous (not commensal of vaginal flora) and endogenous organisms which are commensals of vaginal flora. Microorganisms like Neisseria gonorrhoeae, Chlamydia trachomatis, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Listeria monocytogenes and Trichomonas vaginalis are exogenous organism which causes genital infections by virtue of their biological properties. The endogenous organism like Gardnerella vaginalis, group B streptococci, E coli, Candida can cause infection by their replication dominance which can be influenced by the above mentioned factors and inhibitory or synergistic interrelationships with other microbes among which lactobacilli counts plays a key role.[150]

The common organisms associated with genital tract infections among women are Bacterial Vaginosis, Candidiasis, Gonorrhoea, Chlamydia trachomatis and trichomoniasis. The present chapter looks into the methods for diagnosis of Bacterial Vaginosis, Candidiasis, Chlamydial infection and Gonorrhoea as these are the commonly reported RTIs in India.

2.5.1.a. Methods of diagnosis for Bacterial Vaginosis[151-153]

The vaginal microbiological flora consists of several types of obligate and facultative anaerobic bacteria that are commensal, including Gardnerella Vaginalis, Peptostreptococcus species, Bacteroides species. The Bacterial Vaginosis is a condition caused by overgrowth of organisms mainly Mobiluncus spp, Prevotella spp, Bacteroides spp, Peptostreptococcus, Fusobacterium, Eubacterium spp, Mycoplasma hominis and Ureaplasma urealyticum. In Bacterial Vaginosis,

the lactobacilli count is reduced greatly and there occurs overgrowth of various anaerobes bacteria mentioned above including Gardnerella vaginalis.

Clinical Diagnosis

Bacterial Vaginosis is the most common cause of vaginal discharge among women characterized by fishy odour. The clinical diagnosis (Amsel's criteria) is based on the presence of at least three of the following four criteria mentioned below.

1.Homogeneous white to grey adherent discharge;

2. Vaginal fluid pH of >4.5;

3.Fishy amine odour from the vaginal fluid when mixed with 10% potassium hydroxide (KOH) solution;

4."Clue cells" visible on microscopic examination.

The major limitation of the Amsel's criteria is subjective biases involved in diagnosing Bacterial vaginosis.

Laboratory diagnosis

The common laboratory methods to diagnose Bacterial vaginosis is Nugent and Ison-Hay criteria. Among these laboratory methods to diagnose Bacterial vaginosis the Nugent method is the most evaluated method and is considered the gold standard method.

1. Nugent Method:

The Nugent score uses a system of scoring points allotted to different bacterial morphology, number of different bacteria present in the sample, and amount of lactobacillus present. The method allots scores of 0-10 depending on following points.

Types of organism: large Gram-positive rods (lactobacilli), small Gram-negative or Gram-variable rods (*G. vaginalis* or other anaerobes), and curved Gram negative or Gram-variable rods (*Mobiluncus spp.*).

Quantitative score: The groups are then allotted a score depending on the quantitative weightage given on following points

0 = no morphotype per oil field
1+ = less than 1 morphotype per oil field
2+ = 1 to 4 morphotypes per oil field
3+ = 5 to 30 morphotypes per oil field
4+ = more than 30 morphotypes per oil field.
The scoring system also take into account of lactobacilli morphology present on a smear.

A total score of 0-3 is considered as normal vaginal flora, a score of 4-6 is classified as intermediate flora and score of 7-10 is consistent with a diagnosis of Bacterial vaginosis.

Table 2.5.1. Nugent 's scoring system for diagnosing Bacterial vaginosis

Score	Lactobacillus morphology per vision field	Gardnerella morphology and Bacteroides spp. morphology per vision field	Curved bacteria Gram variable rods morphology per vision field
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	
4	0	>30	

Score:0-3=Suggests normal flora, 4-6= Suggests Intermediate flora, 7-10= Suggests BV

2. Culture:

Since the Bacterial vaginosis is caused by overgrowth of organisms which are commensal of vaginal flora the culture may not be a reliable test to diagnose. Culture is not recommended diagnostic test for diagnosis of Bacterial Vaginosis.

2.5.1.b. Diagnosis of Candidiasis-[150,153]

Candida is a commensal of vaginal flora and under selected circumstances like puberty/pregnancy/uncontrolled diabetes/immunosuppression has a potential to cause disease. Candida is also known to be regulated by oestrogen levels in females. Candidiasis in females is known to be caused by the fungus Candida albicans which contribute to 85% of vaginal infection. The next common type of Candida responsible for the genital infection is C.glabrata with contributes to 15% cases of candidiasis. Since the Candida is a commensal of vaginal flora, the mere gram stain diagnosis may lead to overdiagnosis of infection in absence of symptoms.

Clinical diagnosis of Candidiasis

Clinically, the infection is characterised by increase curdy discharge adherent to vaginal wall on per speculum examination with signs of labial pruritis.

Laboratory diagnosis of Candidiasis.

1. Wet mount preparation- Detection of budding yeast cells by wet mount microscopy examination is known to have a very high predictive value for the diagnosis of Candidiasis.
2. Gram stain smear- The presence of budding yeast cells and pseudohyphae in presence of clinical evident infection is the most preferred method for diagnosing Candidiasis.

3. Vaginal swab culture- Candida can be a commensal of normal vaginal flora, hence the results of culture must be interpreted with caution. The use of culture to diagnose Candidiasis is recommended only if Candidiasis is clinically suspected but microscopy is negative for the organism.

4. PCR Molecular test- The test is highly sensitive for diagnosing Candida spp. The limitation of the test is the overdiagnosis of infection leading to false positive results, in the absence of clinical findings of infection. At presence the test serves no advantage over microscopy test.

Preferred method.

As per WHO guidelines the diagnosis of candidiasis should be established with a combination of clinical features and microscopy of Gram stain smears.

2.5.1.c. Non-Gonococcal infection as a surrogate marker for Chlamydia [154,155,156]

Chlamydia trachomatis is the etiological agent of Chlamydial infection. Among the 13 genotypes (A-K,L1,L2,L3) identified of Chlamydia trachomatis, the genotypes D-K are mainly associated with cervicitis among women.

Clinical diagnosis of Chlamydia

Chlamydial cervicitis is characterised by unhealthy cervix that bleeds on touch with mucopurulent discharge on per speculum examination.

Laboratory diagnosis of Chlamydia

1.Culture

The commonly recommended culture media used for Chlamydia are HELLA 229, monolayer of McCoy. The recommended transport media for the organism is SPG buffer with

antimicrobial inhibitors. Since the organism is not the commensal of the vaginal flora, the advantage of culture in diagnosing Chlamydial infection is high sensitivity and specificity of the test. Culture is a recommended test for diagnosis of Chlamydial infection.

2. Direct immunofluorescence Assay

This test is well evaluated in public health settings and can be undertaken on endocervical swab.

3. Nucleic acid amplification test (NAATs)- The advantage of the test is high sensitivity and specificity, the rapid delivery of the test results and the easy transportation of the specimen with no issue of contamination. At present the limitation of the test is the cost factor, with only few FDA (Food & Drug Agency) approved test. Currently the test is not much evaluated in Indian public health settings.

CDC guidelines for diagnosing Chlamydia in low income countries[154,156]

Chlamydia is a common etiological factor for cervicitis. The accuracy of recommended laboratory test (Culture) to diagnose Chlamydial infection depends on the accuracy of sample collection, selection of transportation media, risk of contamination of the specimen and the technical skill in interpretation of the results. This can be a logistic challenge for low- and middle-income countries like India. The cost factor for the tests also remains a challenge to implement this test at Primary health care level. The increased morbidity associated with genital Chlamydial infection among women, needs this infection to be promptly diagnosed and treated. As per CDC guidelines, since inflammation of cervix (cervicitis) is associated with increased pus cells count, a finding of clinical cervicitis with >10 WBC per high-power field on microscopic examination can be associated with chlamydial infection.

2.5.1.d. Neisseria Gonorrhoea [153-156]

Neisseria Gonorrhoeae is also one of the main causes of cervicitis among women. Along with Chlamydial infection it contributes to the huge burden of urogenital STI/RTIs globally. Among the genotypes (N. lactamica, N. sicca, N. cinerea, N. flavescens, N. subflava, and N. mucosa, N.Gonorrhoeae and N. meningitidis) N.Gonorrhoeae and N. meningitidis are mainly pathogenic in humans and remains the main cause of urogenital infection among women.

Clinical diagnosis of Gonorrhoea

Gonococcal cervicitis is characterised by unhealthy cervix that bleeds on touch with mucopurulent discharge on per speculum examination.

Laboratory diagnosis of Gonorrhoea

1.Culture

Culture remains the gold standard test for genital N.gonococcal infection with high sensitivity and specificity reported. The transport media used is Stuarts or Amies transport medium. The recommended culture media are Thayer– Martin, modified Thayer–Martin media and New York City agar (NYC).

2. Gram stain smear

The Gram stain smear to identify intracellular Gram-negative diplococci within Polymorphonuclear leukocyte (PMNL) is the method of choice for the presumptive diagnosis of N. Gonorrhoeae. In women, however, smears of cervical secretion is reported to detect only 40–60% of culture-positive specimens demonstrating a low sensitivity of Gram stain smear in diagnosing Gonococcal infection. The specificity of Gram stain smear to diagnose Gonorrhoea is reported to be around 80-95% depending on the skill of the microscopist.

3. Nucleic acid amplification test (NAAT)

Recently NAATS nucleic acid have been developed and introduced for detection of specific N. gonorrhoeae DNA/ RNA. These are generally more sensitive than culture in detection of the organism. At present the cost effectiveness of the test remains a challenge for developing countries.

CDC guidelines for diagnosis of Gonorrhoea in low income countries.

A properly prepared Gram stain to identify intracellular Gram-negative diplococci within PMNL is the method of choice for the presumptive diagnosis of N. gonorrhoeae cervical infection with low sensitivity. The specificity of the test for the diagnosis of gonococcal cervical infection is proposed to be high, when evaluated by an experienced technician.

2.5.1.e. Trichomoniasis[153]

The organism is not a commensal of vaginal flora and brings pathogenicity by virtue of its biological properties. It is a recognised cause of vaginal infection among women.

Laboratory diagnosis

1. Wet preparation microscopy-

The demonstration of pear shaped organism with flagella at a higher resolution under a microscope is a hallmark for diagnosing T.Vaginalis infection. The high vaginal swabs eluted into 0.5 ml of sterile saline and a slide prepared with a drop of this saline sample to be observed under the microscope is the recommended method to diagnose this infection. Since the motility of the organism can be lost due to high temperature, the slide should be read within 10 minutes of collection.

2. Point of care- Antigen detection tests.

Various antigen detection assays have been developed to diagnose T.Vaginalis. At present the cost and the performance of the tests vary. The latest generation of these tests namely OSOM Trichomonas Rapid Test (Genzyme Diagnostics, USA) has been reported to have superior sensitivity compared to microscopy.

3. Culture

Since the organism is pathogenic, culture is a recommended test for diagnosing T.Vaginalis.

4. Nucleic acid amplification tests (NAATs)

It is an emerging test, but the cost effectiveness in Indian settings remains questionable.

2.5.2. Molecular tests for Human Papilloma Virus

HPV is a recognised virtual cause for cervical cancer. Testing for HPV, the primary cause for cervical cancer is one of the most widely evaluated biomarker in clinical trials. There are various molecular tests available as mentioned below.[35,157,158]

2.5.2.a. HPV DNA molecular tests are divided broadly into

1. Non amplification technique – Nucleic acids Hybridisation test.

2. Amplification techniques – This group is further classified as

(a) Target amplification, which amplifies the target nucleic acids - PCR

(b) Signal amplification, in which the signal generated from each probe is increased by a compound-probe – Hybrid capture 2 test (HC2), Care HPV, Cervista HR, Cervista HPV 16/18.

(c) Probe amplification, in which the probe molecule itself is amplified --- ligase chain reaction.

Among the molecular tests, four commercially available molecular diagnostic kits have been approved by the United States Food and Drug Administration (US FDA) and have been validated. These tests are

(1) Hybrid Capture 2 (Digene), detects 13 oncogenic HPV. (signal amplification test).

(2) Cervista HPV HR (Hologic), detects14 oncogenic HPVs (signal amplification test).

(3) Cervista HPV 16/18 (Hologic), detects oncogenic type HPV16 and 18. (signal amplification test).

(4) Cobas 4800 HPV (Roche), detects 14 oncogenic HPVs (target amplification test).

Again, among the above molecular HPV DNA test assays, HC2 test and GP5+/6+ PCR has been extensively evaluated in cross-sectional studies.

2.5.2.b. Hybrid capture 2 test [143,144]

HC2 test has probes for high-risk oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 that contribute for cervical carcinogenesis. The test being semi-quantitative, reports the viral load in a specimen which is read in terms of Relative light unit (RLU)/Cut off ratio obtained from the luminometer of the HC2 assay equipment.

Storage of samples

Once samples are collected, they are stored at room temperature for two weeks, at 4°C for one additional week, and at -20°C for up to three months.

Principle of test

The HC2 test is a nucleic acid hybridization assay with signal amplification. The denaturation solution, provided in assay kit is added to the cervical specimen. This breaks the HPV DNA strand. A full B-probe of oncogenic HPV RNA targeting the oncogenic DNA is then added to the specimen. This results in DNA:RNA hybrids being formed. These hybrids formed are then captured onto the surface of a microplate well which is coated with antibodies specific

for DNA: RNA hybrids. A chemiluminescent substrate namely alkaline phosphatase (reagent in the assay kits) is then added to the specimen. The alkaline phosphatase cleavage substrate which is formed. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted which is measured as relative light units (RLU) on luminometer of the machine.

Steps of the test

The reagents required for the test are prepared as per the SOP of Digene manufacturer available in test assay kits.

Step1. Denature HPV DNA

Denaturation reagent which is prepared is added to the testing tube with cervical specimen.

Step 2. Mixing of probes and hybridization

Probe B cocktail mix, having probes for specific oncogenic types is added to the testing tubes. The tubes are then placed in water bath at 65°C for 30 minutes.

Step 3. Step for Hybrid capture

The microplate coated with antibodies specific for DNA:RNA hybrids is numbered for appropriate identification of the patient's sample along with quality control samples as per the test assay kit. The High-risk HPV B probe mix is carefully pipetted into each microplate well. The coated microplate along with the specimen is then placed on a mechanical shaker for 30 minutes.

Step 4: Detection for Hybrid capture

The reagent with the additional antibodies tagged with alkaline phosphatase, is then added to the microplate.

Step 5: Detection, validation, and interpretation

The alkaline phosphatase cleaves the antibody complex formed. The light intensity released due to cleavage by alkaline phosphatase is read using a luminometer which has integrated

computer software. Any sample that emits light as bright or brighter than the light released by a positive control is considered a positive signal for HPV. The results are captured directly into assay specific paper provided by the manufacturer.

Quality control

The HC2 test kits have quality controls samples and assay calibrators that validate the reagents of the test kits and the procedure of the test. The test has two quality control samples, for low risk virus (HPV type 6) and high risk virus (type 16). The assay calibrators have a specific range of % coefficient of verification (% CV) and the test results should fall within the specific range of % CV for the test to be valid, permitting accurate determination of assay cut off value. For all specimens the RLU values are converted into ratio of RLU/cut off value and expressed as RLU/cut off ratio. This is done automatically by the Digene qualitative software and is printed on data analysis report.

Interpretation of the test results.

The test interprets 'HPVDNA detected' for the oncogenic type when the specimen ratio is >/= 1 (RLU/cut off value equal to or more than 1). This corresponds to 5000 or more viral copies present in the cervical sample.

Limitation of the test

A negative test does not rule out HPV infection as samples with HPV viral copies less than the detection limit of the assay can be falsely interpreted as negative test. Antifungal creams, contraceptive jellies, douches can also result in false negative test results. The cross reactivity of probes can occur due to HPV types 6,11,40,42,53,54,55,66 and also plasmids presents in human genital tract leading to false positive test results.

CHAPTER 3
RATIONALE, AIMS AND OBJECTIVES

CHAPTER 3

RATIONALE, AIMS AND OBJECTIVES

3.1. Rationale

There is a significant difference in sensitivity reported for HC2 test to diagnose CIN2+ lesion from developed[39-47] and developing[32,48-53] countries while there is no difference in specificity. There is evidence from India, demonstrating the potential of HC2 test to be accepted as primary screening modality for decreasing cervical cancer mortality in developing countries.[31] Presently, the issue with the HC2 test in implementing as a primary screening test in developing countries like India is its low sensitivity reported.[52,24]

The cross-sectional study conducted by Tata Memorial Hospital (TMH), Mumbai, India,[24] which was a part of multicentric trial, reported 62% sensitivity of HC2 test for CIN2+ lesions after controlling the known factors affecting the test sensitivity. The sample collection technique and transportation of HC2 test were as per Standard operating protocol (SOP) of the Centre. The staff underwent regular rounds of quality control for sample collection methods. There were no verification biases, as all the women enrolled in the study underwent colposcopy. The samples of HC2 tests were processed at Microbiology department which has a NABL accreditation (National Accreditation Board for Testing and Calibration Laboratory). HPVDNA test was processed by HC2 method which has probes for 13 oncogenic HPV types commonly reported from Indian context.[55,99-104] India has a huge burden of undiagnosed and untreated STI/RTIs.[56-58] There is a perception for the role of STIs/RTIs associated with mucopurulent cervicitis in influencing the detection rate of HPV by HC2 test.

The current study explored, if co-infection with mucopurulent cervicitis associated with RTIs is hampering the detection rates of HPV due to cell inadequacy leading to less viral copies

below the detection threshold of the test assay. This may be one of the factors leading to false negative test results affecting the sensitivity of the test.[75,143,144]

3.2. Hypothesis

The results of HPVDNA HC2 testing are affected by presence of blood and mucus associated with cervical inflammation due to RTIs among women co-infected with HPV.

If the study demonstrated, the performance of HC2 test being influenced by the presence of co-infections with RTIs, it would become an important practice to treat co-infections before performing a HC2 test to reduce false negativity of the test results (low sensitivity).

3.3. Aim of the study

To study if concomitant RTIs leading to cervicitis interfere with performance of HPVDNA Hybrid Capture 2 (HC2) testing.

3.4. Objective of study

3.4.a. Primary objective

1. To study whether the presence of clinical cervicitis /or co-infections with lab diagnosed RTIs interfere with the result of HPV DNA testing by HC2 method.

3.4.b. Secondary Objective

1. To determine the prevalence of HPV infection in women with and without clinically diagnosed cervicitis.
2. To determine prevalence of HPV infection in women with lab diagnosed RTIs. (Gonococcal, Non- Gonococcal infections, Bacterial Vaginosis, Candida)

3.5. Ethical clearance

The study was conducted at Preventive Oncology Department of Tata Memorial Hospital (TMH) in collaboration with Department of Microbiology and Department of Obstetrics and Gynecology of H.B.T.M. Collage & Dr R. N. Cooper Hospital (Cooper Hospital). The study protocol was reviewed and approved by the ethics committees and institutional review board of the participating centres respectively. The study was initiated after registering with Clinical Trial.gov. Clinical Trial.gov.NCT02830230.

CHAPTER 4

MATERIAL AND METHODS

CHAPTER 4

MATERIAL AND METHODS

4.1. Study design - Case-Control

4.2. Inclusion criteria

4.2.a. Inclusion criteria for cases

Sexually active (in last 3 months) non-pregnant women aged 30–50 years with clinically diagnosed unhealthy cervix with presence of cervical inflammation that bleeds on touch with mucopurulent or purulent endocervical discharge present on speculum examination irrespective of symptoms.

4.2.b. Inclusion criteria for controls

Sexually active (in last 3 months) non-pregnant women aged 30–50 years with no symptoms pertaining to STI/RTIs (No white discharge P/V, No lower abdominal pain, No urinary symptoms, No dyspareunia, No pruritis vulvae, No post coital bleeding) and clinically no cervicitis or vaginitis on per speculum examination.

4.3. Exclusion criteria

1.The women who received any antibiotics or treatment for reproductive tract infection within the last 4 weeks before enrolment.

2. Women with present or past history of treatment for cervical cancer.

3. Women with only vaginitis without cervicitis for the case arm.

4.Women with history of drug allergy to treating drugs.

(Cefixime, Azithromycin, Secnidazole, Metronidazole, Doxycycline, Fluconazole).

5.Women not willing to follow up.

6.Women currently on antitubercular drugs, or with a known history of immunodeficiency

syndrome.

7.Women who received any HPV vaccination in the past.

4.4. Study setting

The present study was a collaborative study between Tata Memorial Hospital (TMH) with H.B.T.M. Collage & Dr R. N. Cooper Hospital (Cooper Hospital). The women were enrolled from Unit-1 of Obstetrics and Gynecology Department of Dr. R N. Cooper Municipal Hospital and Department of Preventive Oncology Tata Memorial Hospital, Mumbai. All the women enrolled at Cooper Hospital were registered with Preventive Oncology Department for processing of HPV samples. The cervicovaginal slides collected for Gram staining of RTIs were processed at Microbiology Department of Cooper Hospital.

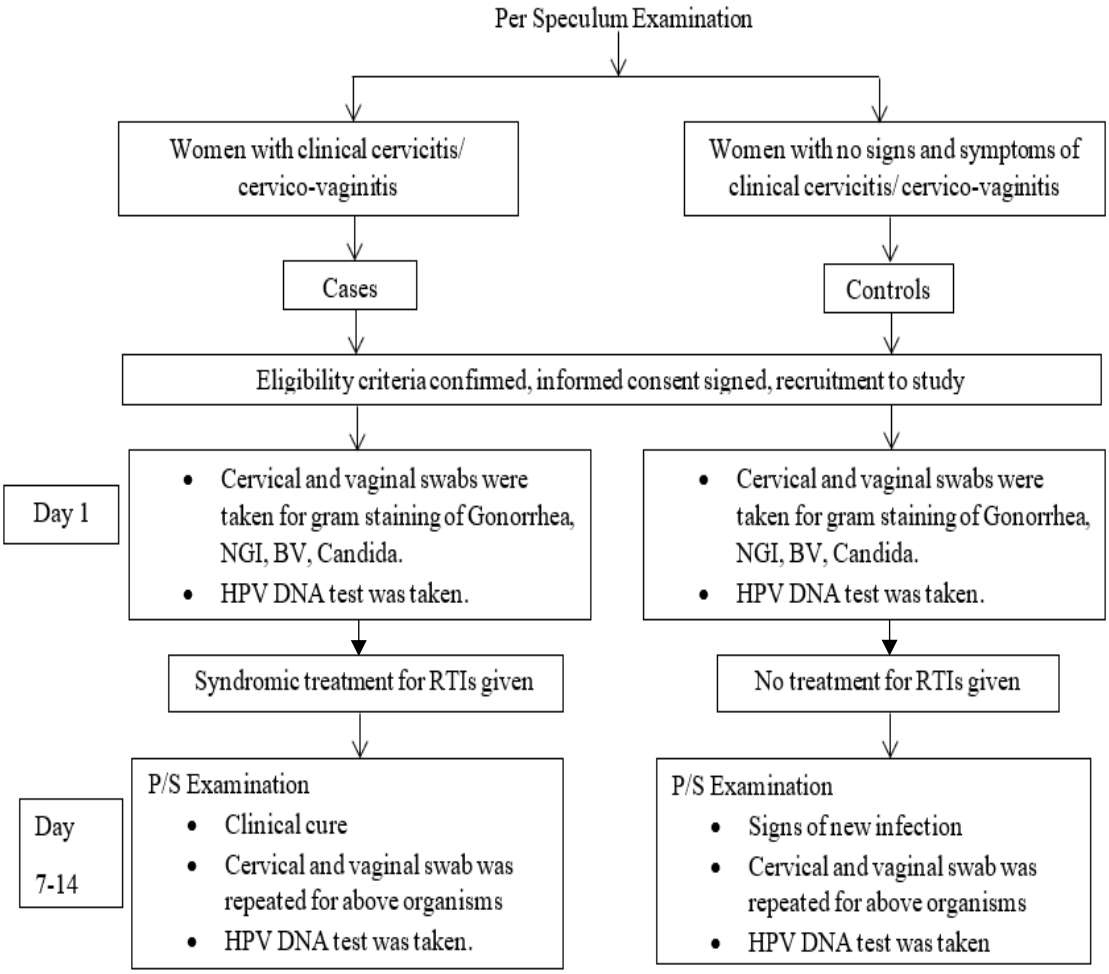
The additional Source of cases to the Department of Preventive Oncology, were women referred for colposcopy in view of unhealthy cervix for further evaluation from Tertiary care hospitals and Private hospitals. The source of controls were asymptomatic women who attended the Department of Preventive Oncology for routine screening of breast and cervical cancer or symptomatic women for breast complains who received an opportunistic screening for cervical cancer.

4.5. Administration of inform consent

Treating doctors administered the informed consent form in the vernacular language to the women participating in the study. The informed consent clearly described the purpose and the process of the study. For women unable to read, the entire informed consent was read out in the vernacular language in the presence of witness. The women were made aware of their right to refuse to participate. They were made to understand the benefits of the study to themselves and to the society. They were assured of the confidentiality that would be maintained in the study.

Any doubts that raised out of the information sheet were completely explained by the treating doctors. The women were also assured that their denial to participate will not affect their treatment and management. Sufficient time was given to each woman to think thereafter. The women willing to participate were enrolled in the study after consenting.

Fig.4.1. Study methodology flow chart



Note- All women enrolled in the study were advised sexual abstinence or use of barrier contraception at baseline visit till the follow up visit.

4.6. Methodology (Fig.4.1)

Baseline evaluation on Day 1

Among women visiting the Departments of Preventive Oncology (TMH) and Department of Obst & Gynaec of Cooper Hospital during the period of August 2016 to August 2018, sexually active, non- pregnant women in the eligible age group of 30-50 years were counselled about the study and invited to participate in the study. All women were then screened for eligibility criteria of the study pertaining to history and findings on per speculum examination.

The women fulfilling the criteria of case/control and willing to follow up were consented for recruitment in the study. After performing per speculum examination, the clinical criteria of case/control were re-confirmed by the two senior doctors (post-doctoral degree in Preventive and social medicine with atleast 1 year of experience and a post-doctoral degree in Obstetrics & Gynecology) in the department. Women with clinical cervicitis on per speculum examination were enrolled as cases and women with no symptoms pertaining to RTIs and no clinical evident genital infection were enrolled as controls.

The women rolled in the study underwent the following steps as a part of study protocol.

Step 1 - A validated structured questionnaire was filled during an interview which consisted of following parts.

- Socio-demographic, reproductive variables, tobacco use, contraceptive history.
- Structured questions to assess the symptoms of RTIs. (White discharge associated with abdominal pain, itching of perineum, burning in micturition, dyspareunia, post coital bleeding/spotting).
- Structured questions to capture the findings of clinical examination and lab diagnosis of STI/RTIs.

Step 2 - During the screening procedure, the women was placed in a lithotomy position and the doctors conducted the examination in the following steps.

- Perineum was inspected for any signs of STIs (genital warts, ulcers, Bartholin cysts) and vaginal discharge.
- Unlubricated bivalve vaginal speculum was inserted and the cervix and vagina were examined for any signs of cervicitis or cervico -vaginitis and the findings were documented.

Step 3 - Cervical swab from ectocervix and endocervix was collected as per the protocol. A vaginal swab was collected from the lateral walls of vagina and posterior fornices.

- The discharge collected by the swabs was evenly spread on a glass slide.

Step 4 – Cervical cells for HPV DNA testing was collected using HC2.

Step 5 - A per vaginal examination was done to diagnose any signs of Pelvic Inflammatory Disease (PID) in terms of any tenderness or any mass in lateral fornices.

Step 6 - The slides were immediately heat fixed, labelled with patient's ID and put in a slide box to be transported to the Microbiology laboratory of Cooper Hospital for Gram staining. The HC2 samples were labelled and transported to Microbiology Department of TMH

The women diagnosed with clinical cervicitis /cervico vaginitis (case arm) were given the Syndromic treatment and partner treatment was advised. The need of partner treatment was strictly counselled to all women & her husband (if accompanying). In case of illiterate women from low socio-economic background, women were advised to carry the empty packets of prescribed drugs at the time of next follow up. The treatment was given free of cost as a part of study protocol.

Instructions for follow up

All the women enrolled in the study irrespective to study arm (case/control), were counselled for follow up and advised the use of barrier contraception (condoms) / sexual abstinence till the second sample collection. No samples were collected during menses. In case a vaginal pessary was advised to women, the women were advised to stop the pessary 3-5 days before she returned for her second sample collection. All women were given a calculated date for follow up.

Follow up evaluation on Day 7-14.

All women enrolled in the study (cases and control) were followed up after 7-14 days.

Step1 – On follow up day, all women were assessed for the use of barrier contraceptives or abstinence, self-treatment and partner treatment compliance. The illiterate women who were asked to follow up with empty packets of prescribed medicines, were assessed for correct administration of drugs and treatment compliance by the treating doctors. Symptomatic women in the case arm at baseline visit were re-assessed for symptomatic cure of the complaints**.** If the women reported to OPD for her second sample collection and was found to have bleeding or vaginal pessary on per speculum examination, no samples were collected and the women were advised to follow up on the recalculated date.

Step 2 - The women underwent per-speculum examination and clinical signs of cervicitis or cervico-vaginitis were reassessed for therapeutic and documented for women enrolled in the case arm (Annexure 1. Table 1). Women in the control arm were assessed for any signs of new infection. If the women reported to OPD for her second sample collection and was found to have bleeding or vaginal pessary on per speculum examination, no sample was collected and the women was advised to follow up on the recalculated date.

Step 3 - A repeat cervical swab and vaginal swab were collected and send for Gram staining for the above-mentioned organisms at the baseline visit.

Step 4 - A repeat, cervical cells for HPV DNA test were collected by HC2 method.

Step 5 - A per vaginal examination was done for women who had signs of Pelvic Inflammatory disease at baseline visit.

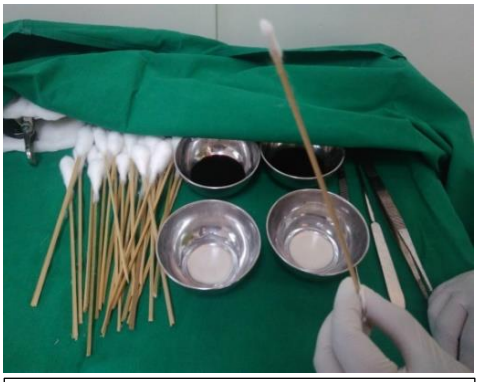
All the women enrolled in the study underwent a Pap smear and a colposcopy evaluation as a part of OPD protocol on baseline visit. The women in the control arm, who were asymptomatic with no signs of RTIs at baseline visit, received treatment on the follow up visit, if detected positive for any pathogenic organism on Gram staining. Women with acetowhite area on colposcopy underwent biopsy. The pathologists were blinded to clinical findings.

Tracking follow ups

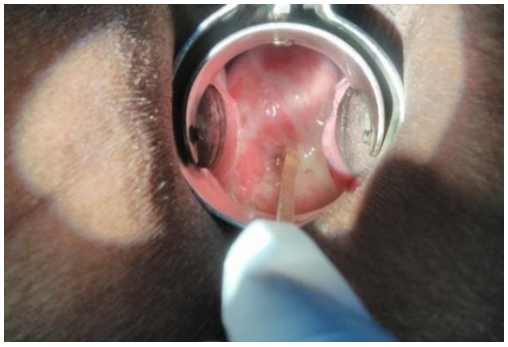
At baseline visit all women enrolled in the study were advised follow up. The follow up date was calculated for the women maintaining the interval of 7-14 days between the sample collections. The record of next follow up visit was maintained in the master sheet along with the telephone numbers. If the woman did not follow up on the allotted date, she was telephonically contacted by medical social worker of the Department of Preventive Oncology. In case of menstrual cycle, the woman was advised to follow up after 5th day of her menstrual cycle or two days after the cycle was over. The medical social worker would allot a new date of follow up to the woman telephonically. In case, if the woman failed to report to OPD after the 1st telephonic call, a second telephonic call was given by the treating doctor and the need of follow up was explained to the woman. The woman was traced for a period of 1 month from the date of her enrollment after which she was labelled as loss to follow up. No samples were collected for woman beyond 1 month even if she reported to OPD. The woman received treatment as per Standard Operating Protocol (SOP) of the Department.

Methodology flow chart

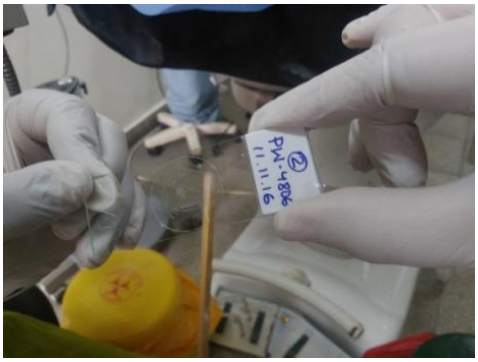
1.Eligibity criteria reconfirmed by two senior doctors after consenting



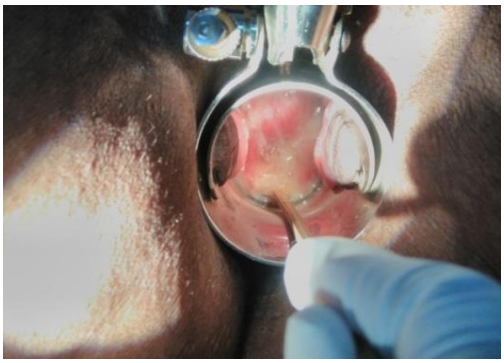
2. Soft cotton stick used to collect discharge



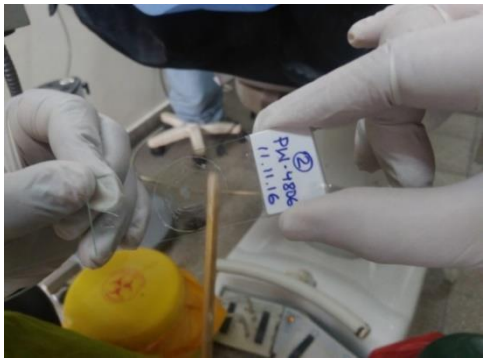
3. Cervical swab taken



4. Cervical discharge uniformly spread on glass slide



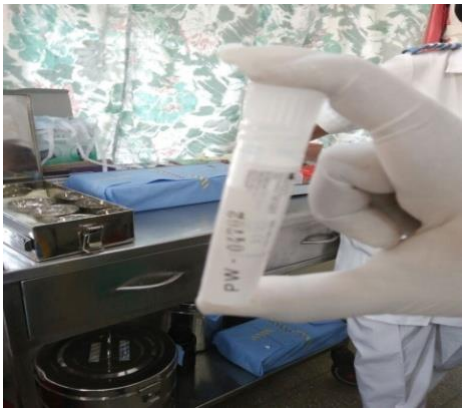
5. Vaginal swab taken



6. Vaginal discharge uniformly spread on glass slide

7. Cervical cells collected by cervical brush provide in HC2 test kit.

8. Brush inserted into transport solution provided by the test kit.



9. HC2 test kit labelled with women's ID.

10. Cervical and vaginal glass slide heat fixed.

11. Heat fixed glass slides stored in slide box ready to be transported.

4.7. Collection, transportation, storage and processing of samples

4.7.a. Cervicovaginal swab for Gram stain.[153]

Soft cotton tip swabs were used to collect vaginal and cervical discharge. For collecting cervical discharge, the soft cotton tip swab was inserted into endocervix and rotated for 5-10 rounds. Vaginal discharge was collected by the same cotton tip swab from posterior fornices and lateral vaginal walls. The discharge was uniformly spread on cervical labelled slide and vaginal labelled slide respectively. The slides were coded with the women's ID code and the date of collection. The slides were immediately heat fixed and put in slide box to be transported to the Cooper Microbiology laboratory for Gram staining. The microbiologist was blinded to clinical findings and HPVDNA test results.

4.7.b. Cervical sample for HPV by HC2 test.[143]

HPV DNA testing was done using the Digene cervical sampler™ (DigeneInc, Gaithersburg, Maryland, USA). After cleaning excess mucus or blood with soft cotton swab stick, the soft cervical brush was inserted in the endocervical os till the outer bristles of the brush were on ectocervix. The brush was then rotated in anticlockwise direction for three and half rotations, and then placed in the transportation medium by breaking the tip of the sampler. These samples were coded with patient's ID code and transported to the Microbiology department of TMH on the same day. The HPVDNA samples collected at Cooper Hospital were transported to Microbiology Department of TMH maintaining a temperature of 15-30^{0}C. The specimen were stored at 4^{0}C if the transportation to laboratory was delayed upto 2-3 weeks. The samples in the Microbiology laboratory were stored at -20 ^{0}C. The test was conducted as per SOP of Microbiology Department of TMH and the instructions of the Digene manufacturer for HC2 assay. The microbiologist was blinded to clinical findings and Gram stain results.

4.8. Laboratory diagnosis for screen positivity

4.8.a. Laboratory diagnosis of RTIs.[150-154,156]

1) Gram stain diagnosis of Candidiasis.

   Gram stain smear showing the presence of budding yeast cells or pseudo hyphae in presence of clinical cervicitis.

2) Gram stain diagnosis of Bacterial Vaginosis (BV)

   Gram stain of the cervico vaginal discharge for diagnosis of BV was done using Nugent criteria. The slides were read at 1000x magnification using oil immersion. The Nugent score uses a system of scoring points allotted to number of different bacteria present in the sample, different bacterial morphology and number of lactobacilli present. The score ranges from 0-10. A total score of 0-3 is considered as normal vaginal flora, a score of 4-6 is classified as intermediate flora and score of 7-10 is consistent with a diagnosis of BV.

   Nugent ‘s scoring system for diagnosing Bacterial Vaginitis.

Score	Lactobacillus morphology per vision field	Gardnerella morphology per vision field	Curved bacteria morphology per vision field
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	
4	0	>30	

Score: 0-3=Suggests normal flora, 4-6= Suggests Intermediate flora, 7-10= Suggests BV.

For the present study the Nugent score of 7-10 was considered positive for BV while the score of 0-6 were considered negative for BV.

3) Gram stain diagnosis of Gonococcal infection

Gram stain showing gram negative diplococci on endocervical smear.

4) Non-Gonococcal infection-surrogate marker for Chlamydial infections.[154,156]

Clinical cervicitis with >10 pus cells/ per high power field on microscopic examination with no other pathogenic organism reported on the endocervical slides (Annexure 1. Table 2).

4.8.b. HPV DNA test.[143]

HPV DNA test was processed by Hybrid Capture 2 method. Cervical samples were classified as being positive for DNA of oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 if the relative light unit (RLU)/cut off ratio reading obtained from the luminometer of the HC2 assay equipment was equal to or greater than 1, corresponding to 5000 or more HPV copies of the virus per ml .

4.9. Pharmacotherapy for Reproductive tract infections.[56,156]

4.9.a. Treatment administered for cervicitis on P/S

Name of drug and Dosage - Tab Cefixime 400 mg orally, single dose.

Tab Azithromycin 1 gm,1 hr before food.

Local Clingen vaginal pessary HS X 6 days

4.9.b. Treatment administered for cervicovaginitis on P/S.

Name of Drug and Dosage – Tab Cefixime 400 mg orally, single dose.

Tab Fluconazole 150 mg after breakfast.

Tab Azithromycin 1 gm,1hr before food.

Tab Secnidazole 2 gm orally, single dose.

Local Clotrimazole 500 mg vaginal pessary HS X 6 days

4.9.c. Treatment administered for women with white discharge with lower abdominal pain.

Name of drug and Dosage - Tab Cefixime 400 mg orally, single dose.

Tab Doxycycline 100 mg twice a day was advised for 14 day

Tab Metrogyl 400mg twice a day was advised for 14 days.

Local Clotrimazole / Clingen vaginal pessary HSX 6 days

4.10. Data Management

The data of women enrolled in the study was recorded on the validated proforma with the unique ID allotted to the women. The Data was entered in the Department of Preventive Oncology, TMH using SPSS-version 24. The quality of data for consistency was checked by the authorized person of the department at a regular interval of time during the study period.

CHAPTER 5

SAMPLE SIZE AND STATISTICAL ANALYSIS

CHAPTER 5.
SAMPLE SIZE AND STATISTICAL ANALYSIS

5.1. Sample Size – (calculated by nMaster 2.0 software)

The prevalence of cervical HPV in general population among Indian women is estimated to be 7.1%.[55] India is expected to have a huge burden of untreated and undiagnosed STIs/RTIs.[57-59]

Cervical inflammation (Cervicitis) caused due to bacterial RTIs is associated with mucopurulent discharge. The hypothesis of the current study was that the mucopurulent cervicitis associated with RTIs is hampering the detection rates of HPV by HC2 test due to cervical cells inadequacy which is one of the proposed reasons for false negative test results of HC2 test. As per hypothesis of the study, after treating cervicitis with antibiotics, we expected detection rates of HPV by HC2 test to increase among women in the case arm if the women were harbouring HPV at baseline visit. The control arm would help us to understand the natural history of cervical HPV among women without clinical cervicitis.

In order to detect 10% difference in HPV detection rates by HC2 test after treatment of cervicitis, with 80% power and error of 5%, the sample size estimated was165 women. Further assuming an attrition rate of 20% for treatment failure and 15% loss to follow up the sample size was worked out to be 254 women. Thus, a total of 508 women, 254 with cervicitis (case arm) and 254 without cervicitis/vaginitis (control arm) were enrolled into the study.

5.2. Statistical application for primary and secondary objectives

The data of the women were captured in SPSS-version 25. Distribution of variables under study were represented by descriptive statistics. Comparisons of baseline characteristics between women enrolled in case & the control arms were assessed by Pearson's $\chi 2$ test & Mann-

Whitney U tests for categorical & continuously-scaled data respectively. Role of risk factors on presence of cervicitis were assessed by using Binary Logistic Regression.

5.2.a. Methodology for statistical evaluation of primary objective

1. The objective of the study was to explore performance of HC2 test in detecting HPV among women with clinical cervicitis/lab diagnosed RTIs before and after treating cervicitis in the case arm.

After controlling the known confounders for false negative test result in the study, Fig 5.1 below shows the HPV outcome status diagnosed by the HC2 test at baseline and follow up visit in case and control arms. At the baseline visit, HPV status among women enrolled in the case arm (mucopurulent cervicitis) or control arm (healthy women with no mucopurulent cervicitis) had two outcome status i.e 'positive for oncogenic HPV' or 'HPV not detected'. On follow up visit, each arm again demonstrated four outcome status in terms of, some women detected HPV positive at baseline visit remained positive (HPV persistence), while in some women among whom HPV was detected at baseline visit demonstrated HPV 'not detected' on follow up visit (no HPV detected/clearance). Some women who were tested negative for HPV at baseline visit were detected HPV positive on follow up visit and some remained negative at baseline and follow up visit. Each arm (case/control) had outcome and suboutcomes on median follow up of 13 days in case arm and 11 days in control arm.

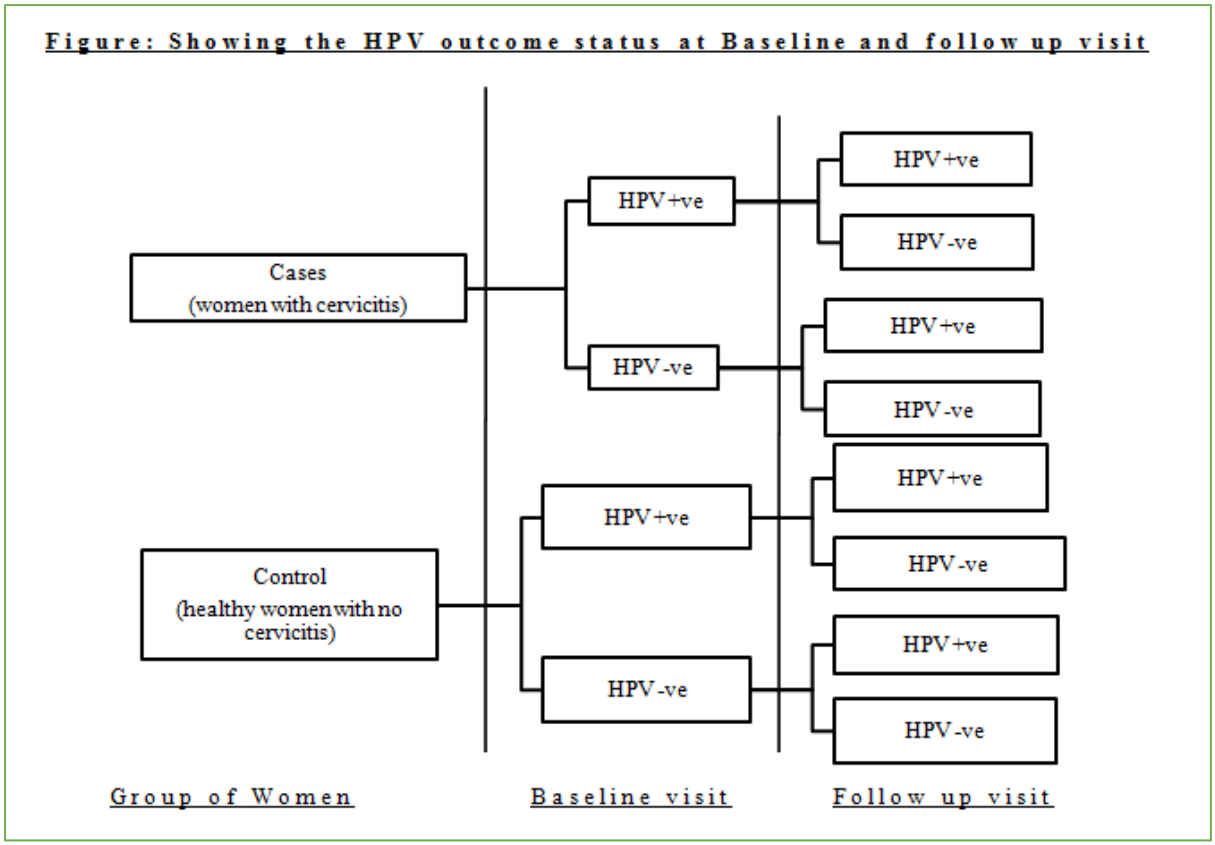


Fig 5.1. HPV status conversion among women enrolled in the study on median follow up of 13 days in Case arm and 11 days in Control arm

The present study demonstrated case/control arm equally dynamic with regards conversions of HPV outcome status captured by HC2 test. There were fix effect/fix variable (clinical cervicitis) which the present study was powered to study its effect before and after treatment on the HPV DNA outcome status (outcome variable) by HC2 test. There were conversions of HPV outcome status also seen within the control arm in a short period of time, hence we expect certain uncontrolled random events /unexplained host factors to have influenced our HPV test results causing subjective variations in test result. The primary objective of the study was analysed using Linear Mix Effect Model (LME). The subjective variations within and between the study arms were accounted for, by including individual ID of women as a random variable in LME model. The LME model took into account the effect of fix variables (clinical cervicitis

and visits) and random variable (individual women ID) on the outcome variable (HPV detected /not detected). The LME model was analysed using R software. The best model with respect to AIC (Akaike information criteria) was selected for predicting the value of parameters included for primary analysis.

Steps for model building

Model – To estimate the effect of clinical cervicitis on HC2 test.

Step 1- A total of 488 women, 243 in the case arm and 245 in the control arm for whom HC2 test results were available at baseline and follow up visits were involved in the analysis.

The variables captured on study proforma

Variables	Coded
HPV status	0= negative
	1= positive
Arm (Case/control)	Case =1
	Control = 0
Visits of women	Baseline= 1
	Follow up =2
Enrolment no	ID of women

Step 2- Table created (Dummy)

Variables recoded for LME model in R software

ID women	Visit*	Arm	HPV status
01	1	0	0
01	2	0	1
02	1	1	1
02	2	1	1
03	1	1	1
03	2	1	0

* Visit represented the change in HPV outcome status.

Step 3- Model represented

HPV status = (case/control + Visits of women) + (ID of women) + (error term)

{outcome} {fixed effect} {random effect}

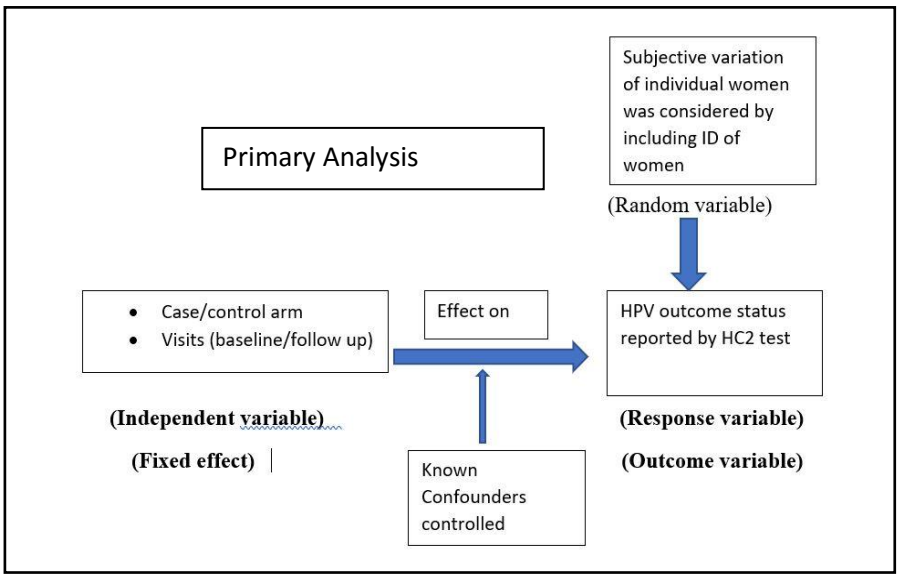


Fig shows the methodology of evaluation of primary objective

5.2.b. Analysis of secondary objective

1.To determine the prevalence of HPV infection in women with and without clinically diagnosed cervicitis.

Presence of HPV infection in women with and without clinically diagnosed cervicitis was represented by Cross tabulation. Association between presence of HPV infection and clinically diagnosed cervicitis was tested by chi square test. Odds of having HPV infection among women with cervicitis as compared to women without cervicitis was calculated by binary logistic regression.

2. To determine the prevalence of HPV infection in women with lab diagnosed RTIs.

Frequency with percentages were reported for women with co-infection of HPV with Candida, Bacterial Vaginosis and Non-Gonococcal infection.

Analysis were performed by Statistical Packages for Social Sciences (Version 25.0, IBM Corp., Armonk, NY) & R version

CHAPTER 6

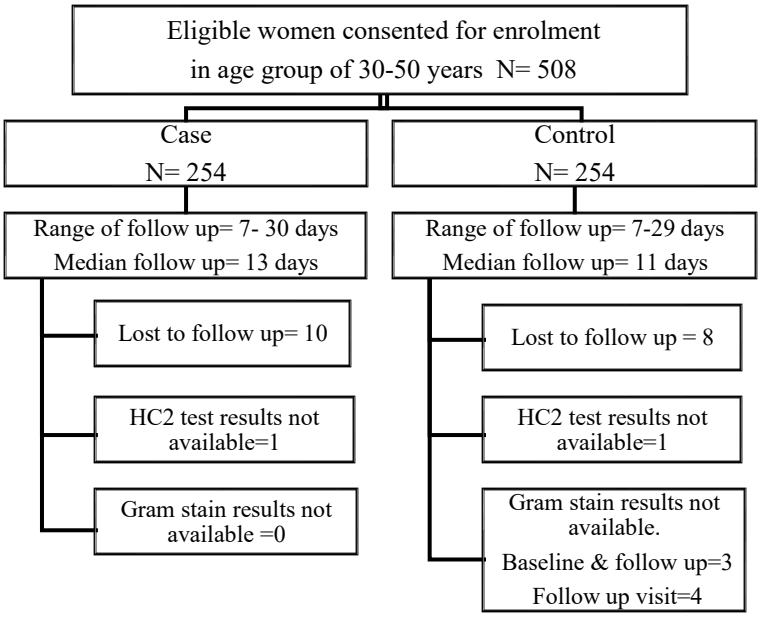
RESULTS

CHAPTER 6

RESULTS

A. Study flow chart

Fig 6.1. Study flow chart showing baseline and follow up status of women enrolled in the study



As shown in Figure 6.1, Among women attending the department of Preventive oncology, sexually active non-pregnant women in the eligible age group of 30-50 years were

counselled about the study and invited to participate in the study. 508 women satisfied for the eligibility criteria who consented for recruitment in the study, among these 254 women satisfying the criteria for cases were enrolled in case arm and 254 women satisfying the criteria for controls were enrolled in control arm. The range of follow up visit in case arm was 7-30 days, median follow up interval was 13 days. Among 254 women enrolled under case arm at baseline visit, 244 women followed up. 10 were lost to follow up and HC2 test result was not available for 1 woman at follow up visit.

Among 254 women enrolled under control arm at baseline visit, the range of follow up visit in this arm was 7-29 days, median follow up interval was 11 days. 246 women followed up and 8 were lost to follow up. HC2 test result was not available for 1 woman at follow up visit. Among women in the control arm, Gram stain results of baseline visit were not available for 3 women and Gram stain result were not available for 4 women at follow up due to loss of slides during transportation.

B. Descriptive analysis

Table 6.1. Distribution of sociodemographic and reproductive characteristics of women enrolled in Case and Control arm at baseline Visit

Variables	Category	Total N=508(%)	Case Arm N=254 (%)	Control Arm N =254(%)
Age	41-50 years	169(33.3)	84(33.1)	85(33.5)
	30 to 40 years	339(66.7)	170(66.9)	169(66.5)
	Median (IQR)*	-	37 (9)	38 (8)
Marital status	Married	484(95.3)	241(94.9)	243(95.7)
	Single/separated/ Widow**	24(4.7)	13(5.1)	11(4.3)
Level of education	Illiterate/primary	56(11.0)	32(12.6)	24(9.4)
	Secondary (upto12th)	204(40.2)	102(40.2)	102(40.2)
	College/university	248(48.8)	120(47.2)	128(50.4)
Religion	Hindu	471(92.7)	239(94.1)	232(91.35)
	Muslim	25(4.9)	11(4.3)	14(5.5)
	Christian/Sikh	12(2.4)	4(1.6)	8(3.1)
Occupation	House wife	326(64.2)	170(66.9)	156(61.4)
	Working women	182(35.8)	84(33.1)	98(38.6)
Monthly family income	Rs 25000 or below	255(51)	119(46.9)	136(53.3)
	Rs 25001 and above	225(44.3)	119(46.9)	106(41.7)
	Income not known	28(5.5)	16(6.3)	12(4.7)
Age of Menarche	Median (IQR)	-	13(1)	13(1)
Age of Marriage	Median (IQR)	-	22 (6)	23(6)
Parity	P0-P1	168(33.1)	86 (33.9)	82 (32.3)
	P2-P3	311(61.2)	150 (59.1)	161(63.4)
	P4+	29 (5.7)	18 (7.1)	11(4.3)
Menopause	Yes	6(1.2)	3(1.2)	3 (1.2)
	No	502 (98.8)	251(98.8%)	251(98.8)

*Interquartile range, **Sexually active

Table 6.1 demonstrates the socioeconomic and reproductive characteristics of women enrolled in the study. Most of the women (66.7%) in the study were in the reproductive age group of 30-40 years. The median age of the women enrolled in case arm was 37 years and 38 years in the control arm. 95.2 % women were married and 4.7% were either ‘single’ or ‘separated’ or ‘widowed’, but

were sexually active for past 3 months. Majority of women in the study were literate (89% class-5 and above) and belonged to Hindu caste (92.7%). 64% of women in the study population were house wives. Women were categorized into high and low income by using median income of Rs25000 as a cut off. There was no difference of income between the study arms. The age of menarche in the study population was 13 years in both the groups. The median age of marriage among women in the case arm was 22 years and 23 years in the control arm. Most of the women (61.2%) had 2-3 live births and were in premenopausal/perimenopausal age group (98.8%). There were no differences in socioeconomic and reproductive determinants for RTI among women in case/control arm except clinical cervicitis among women in the case arm.

Table 6.2. Distribution of contraception use, risk and health seeking behavior for Reproductive tract infection among women enrolled in Case and Control arm at baseline Visit

Variables	Category	Total N=508(%)	Case Arm N=254 (%)	Control Arm N =254(%)	P value OR(CI)
Contraceptive use reported	Regular barrier user	60(11.8)	28(11)	32 (12.6)	0.413
	Other contraceptive user*	172 (33.9)	93(36.6)	79 (31.1)	
	No contraceptive users	276 (54.3)	133(52.4)	143(56.3)	
Number of sexual partners	Single	481(94.7)	234(92.1)	247(97.2)	0.014 3.0 (1.3-7.3)
	Multiple	27(5.3)	20(7.9)	7(2.8)	
Tobacco use reported	Non user	476(93.7)	238(93.7)	238(93.7)	1.000
	User	32(6.3)	16(6.3)	16(6.3)	
Previous history of RTI** treatment received within 1 year	No previous history	330(65)	129(50.8)	201(79.1)	0.001 3.6 (2.5-5.4)
	Previous history	178 (35)	125(49.2)	53(20.9)	
Partner notification reported among women with previous history of RTIs	Yes	28(5.5)	19 (7.5)	9 (3.5)	0.765
	No	150 (29.5)	106 (41.7)	44 (17.3)	
	N/A***	330 (65)	129(50.8)	201(79.1)	
Health seeking behavior for RTI treatment	Government hospital	53(10.4)	36(14.2)	17(7%)	0.662
	Private hospital	125(24.6)	89(35)	36(14.2)	
	N/A***	330(65)	129 (50.8)	201(79.1)	

*Tubectomy/Oral contraceptive pills/Intrauterine contraceptive device/Vasectomy.

Reproductive tract infection. * Not applicable

As seen in Table 6.2, among the study population 11.8% of women reported regular use of barrier contraception and 33.9% reported the use of other contraceptive methods. Majority of women (54.3%) in the study population reported no use of any contraception. There was no difference in the patterns of contraceptive use between women in the case or control arm. A total of 94.7% women in the study population reported single sexual partner while 5.3% women reported having multiple sexual partners. The history of multiple sexual partner was reported 3 times higher among women in case arm than in the control arm. Tobacco use was reported in 6.3% women in the study population with no difference between case and control arms. Among the study population, 35% of women reported a past history of RTI treatment. The past history of RTI treatment reported was 3.6 times higher in women in the case arm than the control arm. Among the women who reported treatment received for RTIs in the past, majority of women 84.7% (n=150/178) reported, no partner notification. No partner notification was reported higher in women among the case arm (41.7%) than the control arm (17.3%), though the difference was not statistically significant. The study demonstrated, majority of women 70.2% (n=125/178) sought treatment for RTIs from private practitioner versus government hospital. There was no differences between the case and control arm in reporting health seeking behavior for RTIs.

Fig 6.2. Common presenting complaints of Reproductive tract infections among symptomatic women enrolled in case arm at baseline visit

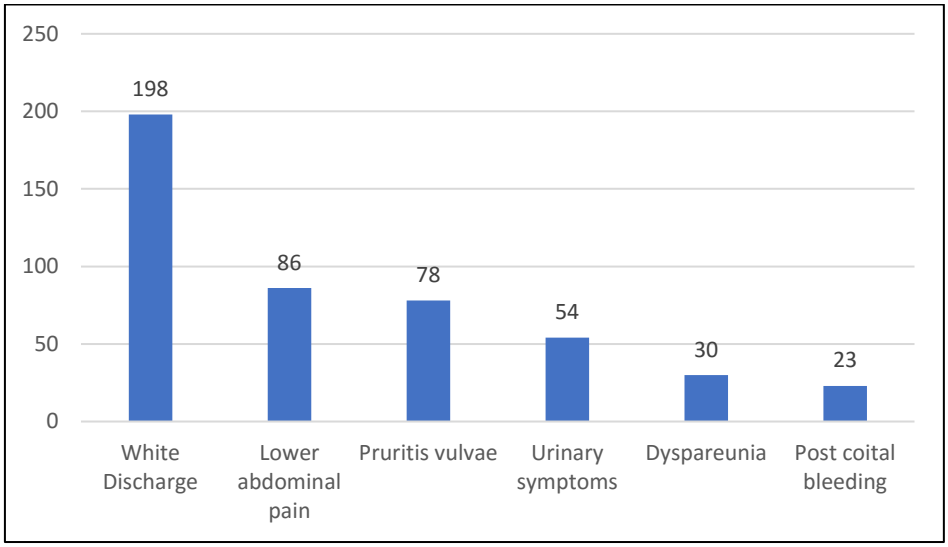


Table 6.3. Common presenting complaints of Reproductive tract infections among symptomatic women in case arm (N=204)

White discharge P/V	Lower abdominal Pain	Pruritis vulvae	Urinary symptoms	Dyspareunia	Post coital bleeding
198(97%)	86 (42.2%)	78 (38.2%)	54 (26.5%)	30 (14.7%)	23(11.3%)

* Data shows women with atleast one symptom of reproductive tract infection**.**

Fig 6.2 and Table 6.3 demonstrates, among 204 symptomatic women in the case arm at baseline visit, the most common symptom was white discharge (97%) followed by lower abdominal pain (42.2%) and pruritis vulvae (38.2%). 26.5% women complained of burning in micturition, 14.7% reported history of dyspareunia and 11.3% had post coital bleeding.

Table 6.4. Clinical sign and symptoms of Reproductive tract infection among women enrolled in case arm at baseline visit

Clinical signs and symptoms		N=254 (%)
Symptoms pertaining to RTIs	Symptomatic	204 (80.3)
	Asymptomatic	50 (19.7)
Clinical findings on speculum examination	Cervicitis	80(31.4)
	Cervicovaginitis	174(68.5)

Among the women enrolled in case arm with clinically evident cervicitis on per speculum, 80.3% women were symptomatic at baseline visit, while 19.7% were asymptomatic. On per speculum examination 31.4% had cervicitis and 68.5% women had cervico-vaginitis (Table-6.4).

Table 6.5. Burden of Reproductive tract infection reported on Gram stain among women at baseline visit

Category of screen positive criteria for RTIs on Gram stain	**Category of women enrolled in study**		
	Case Arm* N=254(100%)	**Control Arm* N=251(100%)**	**Total N= 505*(100%)**
Overall positivity rates of RTIs**	239 (94)	10 (4)	249 (49.3)
Distribution of organism reported on Gram stain among positive women for RTIs (criteria for screen positive for RTI infection)			
Bacterial vaginosis (Nugent score> 7)	95 (37.4)	10 (100)	105 (20.8)
Candidiasis **(**Presence of pseudohypae on the Gram stain in presence of clinical cervicitis)	15 (5.9)	0	15 (3)
Bacterial vaginosis with Candidiasis (Nugent score> 7 and presence of pseudohypae on the Gram stain in presence of clinical cervicitis)	17 (6.6)	0	17 (3.4)
Non Gonococcal Infection (Clinical cervicitis and Gram stain showing > 10 pus cells/HPF and no other pathogen reported)	112 (44.1)	0	112 (22.2)

*****The above table includes women with Gram stain results available at baseline visits.
**Reproductive tract infection

The overall positivity rate of RTIs in the study population was 49.3%. The positivity rates for at least one RTI was 94% among women enrolled in the case arm versus 4% in the control arm at baseline visit. Among women in the case arm, 37.4% had BV reported on Gram stain, 5.9% had Candidiasis, 6.6% had multiple infections of BV with Candidiasis and 44.1% women reported to have NGI on Gram stain smear. Among the 10 women diagnosed with RTI in the control arm, all had BV reported on the Gram stain smear. No Gonorrhoea was reported on Gram stain smears among the women in the study population (Table-6.5).

Table 6.6. Pap cytology results of women enrolled in case and control arm at baseline visit (as a part of OPD protocol)

	Category of Pap smear by Bethesda classification	**Case Arm N=254(%)**	**Control Arm N=254(%)**
1	Inadequate for evaluation	5 (2)	5(2)
2	Normal/Inflammatory smear with no infection	89 (35)	148(58.3)
3	Total BV Positive smear reported	138 (54.3)	91(35.8)
4	Total Epithelial cell abnormalities reported	46 (18.1)	12(4.7)
	ASCUS[Ŧ]	13 (5.1)	4(1.5)
	LSIL[ŦŦ]	26 (10.2)	8 (3.1)
	HSIL[ŦŦŦ]	7 (2.8)	0
5	Epithelial cell abnormalities with BV (ASCUS, LSIL, HSIL) [Ŧ]	31 (12.2)	8(3.1)
6	Total Candida /Trichomoniasis reported	7 (2.8)	6(2.4)

[Ŧ] Atypical squamous cells of undetermined significance, [ŦŦ] Low grade squamous intraepithelial lesion, [ŦŦŦ] High grade intraepithelial lesion.

Table 6.6 shows the distribution of RTIs and epithelial cell abnormalities on Pap cytology among women enrolled in the study. All the women enrolled in present study received Pap cytology as a part of departmental protocol.

Among 254 women in case arm, 2% women had Pap cytology reported inadequate for evaluation. 89 (35%) women had normal/inflammatory smears reported without pathogen. Pap cytology reported BV infection in 54.3% and epithelial cell abnormalities in 18.1% women in case arm. Overall in case arm (254),12.2% women had epithelial cell abnormalities with BV infection reported on Pap smear. Candida or Trichomoniasis infection were reported in 2.8% women.

Among 254 women in the control arm in absence of clinical infection, 2% had Pap cytology reported inadequate for evaluation. Majority of women (58.3%) had normal/inflammatory smears reported without pathogen. Pap cytology reported BV in 35.8% and epithelial cell abnormalities in 4.7% women. Overall in the control arm (254), 3.1% women had epithelial cell abnormalities with BV and 2.8% had Candida. There was significant difference in epithelial cell abnormalities reported on Pap cytology among women in case arm versus control arms (p=0.001).

Table 6.7. Prevalence of HPV among women at baseline visit

		Case Arm N= 254(%)	**Control Arm N=254(%)**	**Total women. N=508(%)**
HPV by HC2 Day-1	HPV negative	218(85.8)	241(94.9)	459(90.4)
	HPV positive	36 (14.2)	13 (5.1)	49 (9.6)

As seen in Table 6.7, the overall HPV prevalence in the study population was 9.6% at the baseline visit. The prevalence rates of HPV infection among women in the case arm was 14.2% as compared to 5.1% women in the control arm.

Table 6.8. Distribution of histopathology reports among women with acetowhite lesion on colposcopy (as a part of OPD protocol)

	Case Arm* N= 56 (100%)	Control Arm* N= 26 (100%)	Total N=82(100%)
Benign lesions			
Squamous Metaplasia	26 (46.4)	22 (84.6)	48 (58.5)
Cervicitis	21(37.5)	3 (11.5)	24 (29.2)
Precancerous lesions			
CIN-1	1(1.8)	1(3.8)	2 (2.4)
CIN-2& CIN-3	8 (14.3)	0	8 (9.7)

* Data includes women who underwent colposcopy directed biopsy for acetowhite lesion (part of OPD protocol).

All the women underwent colposcopy examination as a part of OPD protocol. Among biopsy taken for 56 women in the case arm 46.4% had squamous metaplasia & 37.5% had cervicitis. 1.8% and 14.3% women had CIN-1 and CIN-2&3 lesions on histopathology respectively. Among the biopsy taken for 26 women in the control arm 84.6% reported squamous metaplasia, 11.5 % reported cervicitis in the absence of signs of cervicitis and 3.8% reported CIN-1 lesion reported on histopathology. There were no women detected with invasive cervical cancer lesion (Table 6.8).

Table 6.9. Compliance to syndromic treatment, partner treatment and use of barrier contraception reported among women on follow up visit

Category	Case Arm* N=244(%)	Control Arm* N= 246(%)
Compliance to self-treatment	236 (96.7)	N/A**
Compliance to partner treatment	231(94.7)	N/A**
Compliance to barrier contraception/sexual abstinence	231 (94.7)	199 (80.9)

*Table includes the data of women who followed up.

** Not applicable as the control arm received was no treatment.

Women in the case arm were given treatment for RTIs and partner treatment was advised at baseline visit. Good compliance to advised treatment (96.7%) and partner treatment (94.7%) was demonstrated in case arm on follow up visit. All women enrolled in the study were advised use of barrier contraception/sexual abstinence irrespective to the arm enrolled. Compliance to sexual abstinence/ barrier contraception was reported by 94.7% and 80.9% women in case and control arms respectively on follow up visit (Table 6.9).

Table 6.10. Therapeutic response to lab diagnosed Reproductive tract infection among women enrolled in the study

Category	Case Arm (Intervention given) * N= 244 (100%)		Control Arm (No Intervention given) * N=242(100%)	
	Baseline Visit	Follow up visit	Baseline Visit	Follow up visit
Prevalence of Lab diagnosed RTIs	230 (94.3)	21 (8.6)	9 (3.7)	16 (6.6)
Distribution of organism reported on Gram stain among positive women for RTIs (criteria for screen positive for RTI infection)				
Bacterial vaginosis	107 (43.8)	9 (3.7)	9(3.7)	16 (6.6)
Non-Gonococcal infection	110 (45)	10 (4.1)	0	0
Candidiasis	30 (12.3)	2 (0.8)	0	0

*The above data includes women who followed up and had gram stains results available for baseline and follow visits to assess the therapeutic effect of syndromic treatment for individual pathological agent.

Table 6.10, demonstrates the therapeutic effect of syndromic treatment on Lab diagnosed RTIs. Among women who followed up, Gram stain results of baseline and follow up visit were available for 244 women in case arm and 242 women in the control arm. Among 244 women in case arm who received treatment for RTIs, the burden of RTIs were significantly reduced from 94.3% to 8.6% on follow up visit. The prevalence of BV was reduced from 43.8% to 3.7%, NGI reduced from 45% to 4.1% and Candidiasis reduced from 12.3% to 0.8%. Among 242 women in control arm who received no treatment for RTIs, the prevalence of asymptomatic BV increased from 3.7% to 6.6% at follow up visit in the absence of clinically evident genital infection. Women in the control arm diagnosed with BV received treatment on follow up visit.

Table 6.11. Distribution of HPVDNA test results at baseline and follow up visit.

	Case Arm (N=243) *				Control Arm (N=245) *			
HPV test results	**Positive N (%)**		**Negative N (%)**		**Positive N (%)**		**Negative N (%)**	
Baseline visit	35(14.4)		208(85.6)		13 (5.3)		232 (94.7)	
HPV test results	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Follow up visit	26 (74.3)	9 (25.7)	11 (5.3)	197 (94.7)	7 (53.8)	6 (46.2)	4 (1.7)	228 (98.3)

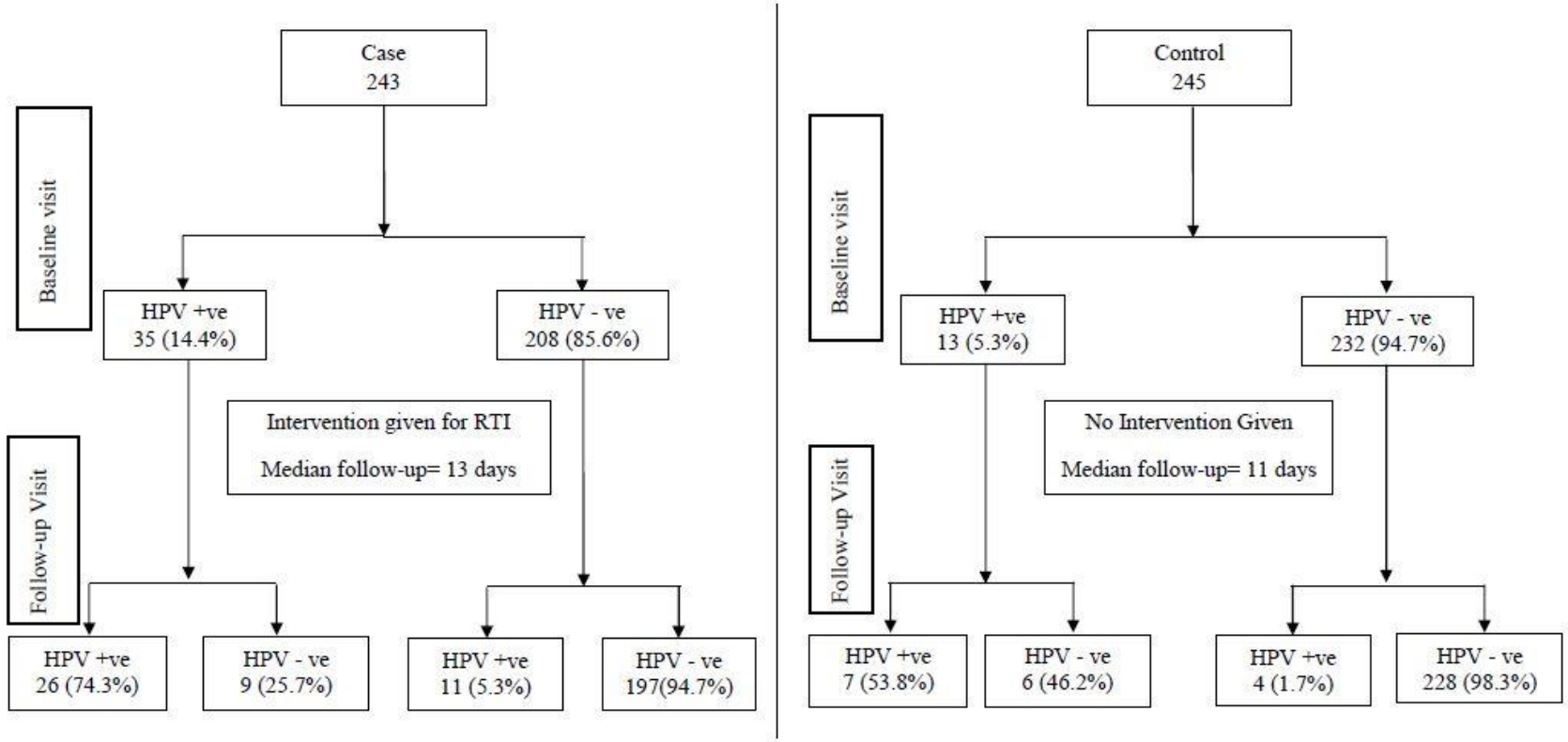
*The above data includes women who had HC2 results available for baseline and follow visits (Median follow up for Case arm was 13 days and for Control arm was 11 days)

As shown in Table 6.11 and Fig 6.3, HPV test results of baseline and follow up visits were available for 243 in the case arm and 245 in the control arm at follow up visit.

Among 243 women in the case arm, 14.4% (n=35) were detected HPV positive and in 85.6 % (n=208), HPV was not detected at baseline visit. Among 35 women with HPV positive at baseline visit, 74.3% (n=26) remained HPV positive and in 25.7% (n=9) HPV was not detected at follow up visit. Among 208 HPV negative women at baseline visit, in 5.3% (n=11) HPV was detected at follow up visit after treating cervicitis while 94.7% (n= 197) remained HPV negative.

Among the 245 women in the control arm, 5.3% (n=13) women were detected HPV positive and in 94.7% (n=232) HPV was not detected at baseline visit. Among the 13 women with HPV positive at baseline visit, 53.8% (n=7) remained HPV positive and 46.1% (n=6) HPV was not detected on follow up visit. Among 232 HPV negative women at baseline visit, in 1.7% (n=4) HPV was detected at follow up visit, while 98.3% (n=228) remained HPV negative at follow up visit.

Fig 6.3. HPVDNA test results among women at baseline and follow up visit.



***The above Fig includes women who followed up and had HC2 results available for baseline and follow visit**

Table 6.12. HPVDNA status at follow up visit assessed by RLU titers at baseline visit

RLU titers of HPV positive test results	HPVDNA status among women at follow up visit						
	Case Arm N= 46			Control Arm N= 17			Total N=63
	BL (+) FU (+) N= 26 (100%)	BL (+) FU (-) N= 9 (100%)	BL (-) FU (+) N=11 (100%)	BL (+) FU (+) N= 7 (100%)	BL (+) FU (-) N= 6 (100%)	BL (-) FU (+) N= 4 (100%)	63 (100%)
1-10 RLU/CO	6 (23%)	7 (77.8%)	7 (63.6%)	1 (14.3%)	5 (83.3%)	2 (50%)	28 (44.4%)
11-100 RLU/CO	6 (23%)	2 (22.2%)	1 (9.09%)	4 (57.1%)	1 (16.7%)	1 (25%)	15 (23.8
Above 101 RLU/CO	14 (53.8%)	-	3 (27.3%)	2 (28.6%)	-	1 (25%)	20 (31.7%)

BL (+) FU (+) - HPV positive at baseline and follow up visit. (repeat test positive)

BL (+) FU (-) - HPV positive at baseline and not detected on follow up visit (repeat test negative)

BL (-) FU (+) - HPV negative at baseline and positive on follow up visit. (Assumed false negative test results at baseline)

RLU/CO - Relative Light Unit/ Cut off ratio (RLU titers)

Table 6.12 demonstrates, among women who followed up, 46 in case arm and 17 in control arm (63) suffered at least one episode of oncogenic HPV infection during the study period. Among the 46 women in case arm, 26 of them had repeat test positive for HPV infection. Among these 26 women (6/26) 23% had RLU titers between 1-10, (6/26) 23% had RLU titers between 11-100 and (14/26) 53.8% women had RLU titers above 100 at baseline visit. Among the 46 women 9 were negative for HPV infection at follow up visit. Among these 9 women (7/9) 77.8% and (2/9) 22.2% had RLU titers in range of 1-10 and 11-100 respectively. It was observed that none of the women demonstrating repeat test negative for infection on follow up visit had RLU titers above 100 at baseline visit. Among the 11 of 46 women in case arm who were initially HPV negative at baseline visit and were detected HPV positive after treatment of cervicitis, majority (7/11) 63.6% of women demonstrated RLU titers between 1-10, while (1/11) 9% were of RLU titers between 11-100 and (3/11) 27.3% had RLU titers above 100 on follow up visit.

Among the women in control arm, 17 women suffered at least one episode of HPV infection during the study period. Among these 17 women, **7** were positive for HPV infection at follow up visit. Among these 7 women, (1/7) 14.3%, (4/7) 57.1% and (2/7) 28.6% had RLU titers between 1-10, 11-100 and titers above 100 respectively at baseline visit. Among the 6 women, who were with negative test results at follow up visit (5/6) 83.3% and (1/6) 16.7% had RLU titers in range of 1-10 and 11-100 respectively. It was also observed that none of the women demonstrating clearance of infection on follow up visit had RLU titers above 100 at baseline visit. Among the women (n=4/17) who were initially HPV negative at baseline visit and were detected HPV positive in control arm, (2/4) 50% demonstrated RLU titers between 1-10, (1/4) 25% were detected with RLU titers between 11-100 and (1/4) 25% had RLU titers above 100 on follow up visit.

C. Primary Analysis

1.To study whether the presence of clinical cervicitis or co-infections with Lab diagnosed RTIs interfere with the result of HPV DNA testing by Hybrid Capture 2 method.

Table 6.13. Distribution of women according to HPV DNA test results at baseline and follow up visit

HPVDNA Conversions	Case Arm N=243(%)*	Control Arm N=245(%)*
BL (+), FU (+) (Repeat test positive)	26(10.7)	7(2.9)
BL (+), FU (-) (Repeat test negative)	9(3.7)	6(2.4)
BL (-), FU (+) (Assumed false negative)	11(4.5)	4(1.6)
BL (-), FU (-) (No conversions)	197(81.1)	228(93.1)

*The above Fig includes women who followed up and had HC2 results available for baseline and follow visits

BL (+), FU (+) = HPV detected at baseline and follow up visit. (Repeat test positive)

BL (+), FU (-) = HPV detected at baseline visit and not detected at follow up visit.

(Repeat test negative)

BL (-), FU (+) = HPV not detected at baseline visit and detected at follow up visit.

(Assumed false negative at baseline)

BL (-), FU (-) = HPV not detected at baseline and follow up visit. (No conversions)

Table 6.13 shows the overall distribution of women with HPV test results at baseline and follow up visit at median interval of 2 weeks. The overall repeat test positive result for HPV in the study population was 10.7% in case arm versus 2.9% in the control arm. The overall women with repeat test negative result were 3.7% in case arm and 2.4% in the control arm. The conversion of HPV test results from HPV not detected at baseline visit and detected at follow up visit was 4.5% after treatment of cervicitis in case arm, whereas 1.6% were detected positive among women without cervicitis. 81.1% women in case arm and 93.1% women in control arm remained negative at baseline and follow up visit.

Table 6.14: Estimates obtained through Linear Mix Effect model for primary objective

Sr.no	Parameters	Regression Estimates	Standard error	P value	Regression Estimates (Confidence interval)
1.	Intercept	0.04	0.02	0.040	0.00-0.09
2.	**Intervention (case/control)**	**0.09**	**0.02**	**0.001**	**0.05-0.14**
3.	Visit (BL/FU)*	0.00	0.01	1.000	-0.02- 0.02

* Baseline visit / Follow up

There were conversions of HPV outcome status demonstrated in case and control arm as demonstrated in Table 6.13. Since the conversions were not expected in the control arm, we needed to account for the uncontrolled host factors (innate immunity, RLU titers of the HC2 test assay) and random events (related to sexual history reported by women) that would have caused the HPV conversions in both arms. To know the true effect of our intervention on the outcome status of HPV captured by HC2 test, the subjective variability at individual level along with fix variable (clinical cervicitis in the present study) were taken into account by applying Linear Mix Effect model. The model as shown in Table 6.14 estimated the effect of clinical cervicitis on test results of HC2 at follow up visit. The p value observed was statistically significant (p=0.001). The model demonstrated that intervention given for clinical cervicitis played an influencing role on modifying (increasing) the detection rates of HPV by HC2 test.

D. Secondary analysis

1.To determine the prevalence of HPV infection in women with and without clinically diagnosed cervicitis.

2.To determine the prevalence of HPV infection in women with lab diagnosed RTIs at baseline visit.

Table 6.15. Prevalence of the HPV infection among women enrolled in case and control arms at baseline visit

		Case Arm N=254(%)	Control Arm N=254(%)	Total N=508(%)	OR (95% CI)	p value
HPV by HC2 Day-1	HPV negative	218(85.8)	241(94.9)	459(90.4)	3 (1.5%-5.9%)	0.001
	HPV positive	36(14.2)	13 (5.1)	49 (9.6)		

The overall prevalence of HPV in the present study was 9.6% at baseline visit as seen in Table 6.15. The prevalence of HPV infection among women in case arm was 14.2% as compared to 5.1% among women in the control arm. The risk for HPV infection was observed 3 times higher in women in the case arm as compared to women in control arm (OR=3, CI-1.5%-5.9%).

Table 6.16. Prevalence of HPV infection in women with lab diagnosed RTIs at baseline visit

Organism reported	HPV positive N = 31	HPV negative N= 218	Total* N= 249
BV**	14 (13.3)	91 (86.7)	105(100%)
Candidiasis	0	15 (100)	15(100%)
BV with Candidiasis	2 (11.8)	15 (88.2)	17(100%)
NGI***	15(13.4)	97 (86.6)	112(100%)

*The above table includes women with Gram stain results available at baseline visit enrolled in Case and Control arm

** Bacterial Vaginosis, ***Non-Gonococcal infections.

Among the total 105 BV reported at baseline visit, the prevalence of HPV infection reported was 13.3% (n=14). There were no HPV reported among women diagnosed with Candidiasis. Among multiple infections on Gram stain with BV & Candidiasis, the prevalence of HPV was 11.8% (n=2) and prevalence of 13.4% (n=15) were reported among women diagnosed with NGI (Table- 6.16). The overall prevalence of HPV among women diagnosed with RTIs was 12.4%.

CHAPTER 7

DISCUSSION

CHAPTER 7

DISCUSSION

The molecular HC2 test is a FDA approved test extensively validated in various cross-sectional studies worldwide.[143,144] The test identifies women who may be at risk of developing cervical cancer due to the presence of oncogenic HPV. The Multicentric cross-sectional study from India demonstrates the potential of HC2 test to be accepted as a primary screening test in the near future.[31] The advantages of the HPV molecular test-based screening apart from high sensitivity and needing less screening rounds are – a) Minimal resources and manpower needed for performing the test. b) Potential of the samples being self-collected. c) The reproducibility and standardization of the test. While the developed countries are adopting HC2 test as a primary screening test in their national screening programs as per the recommendation,[36,37,38,148] there are still concerns on low sensitivity of the HC2 test reported from the developing countries including India.[149]

The present study was powered to evaluated the probable role of RTIs resulting in false negative HC2 test results, in an attempt to explore low sensitivity of the test reported from Indian context.

1- The socioeconomic and reproductive characteristics of the women enrolled in the study.

The present study targeted women in the age group between 30-50 years, as women in this age group are expected to be sexually active, making them prone for RTIs. Majority of women in the study were in age group of 30-40 years, the median age being 37 and 38 years in case and control arm respectively. This age contributes to the highest burden of RTIs among women globally.[57] As per the natural history of HPV, the women in the age group of 30-50 years have more chances of being diagnosed with cervical precancerous and cancerous lesion due to

persistence of oncogenic HPV.[12,85,87] Screening for cervical cancer with HPVDNA molecular test in the age group of 30 and above is expected to demonstrate maximum gains in terms of reducing cervical cancer mortality.[148] The risk factors for RTIs including HPV infections among women reported are, sexually active women, lack of education and low socioeconomic status. Early age of menarche and marriage, limited or no contraceptive knowledge makes women vulnerable to repeated pregnancies and recurrent RTI infections which are the recognized cofactors for persistent HPV infections.[57,59,63,123,127,128,159] There is evidence for increase parity to modulate the progression of HPV infection to cervical cancer.[15,16] The pregnancy related hormones cause cervical ectropion leading to the large transformation zone, being exposed on ectocervix where the cells are actively dividing. The actively dividing cells at the transformation zone makes women prone for acquisition of HPV infections. Repeated pregnancy causing wear and tear of cervical tissue and poor genital hygiene makes women venerable to RTIs. The RTIs causing cervicitis (cervical inflammation) play a major role in persistence and progression of HPV infection to precancerous and cancerous lesion of cervix.[13,14,118,119]

In the current case-control study, there was no difference in the distribution of determinants of STI/RTIs among women enrolled in both study arms except clinical cervicitis in the case arm (Table 6.1).

2- Determinants of RTIs reported in the study

In the current study (Table 6.2) it was observed that only 11.8% women reported regular use of barrier contraception (Condoms) and 54.3% reported no use of any contraception methods. The history of multiple sexual partners was reported significantly higher among women in the case arm than in the control arm. These findings are known risk factors for RTIs and HPV infection.[57,128] Tobacco is an independent predictor for cervical carcinogenesis due to its ability

to cause mutations in DNA of cervical cells basically due to the nicotine content. It is also a recognized cofactor for persistent HPV infection among women.[15,115] In the present study 6.3% women reported use of tobacco (mainly masheri) with no difference in usage demonstrated among the study arms.

The previous history of RTIs was reported among 35% (n=178/508) women in the study and was observed to be significantly higher among women in the case arm. In current study, we could elicit the history of partner's treatment among the 178 women who reported previous history of RTI treatment. 84.3% (n=150/178) of the women who suffered previous episode(s) of RTI reported no partner treatment. The lack of concurrent treatment of the partner's either due to gap in knowledge of the service providers or lack of cooperation from partners in Indian communities are reported to be the contributing factors for the above findings in the existing STI programs run by the government.[59,128] This findings also demonstrates that the male partners forms the neglected population for STI screening & treatment in the developing countries like India.

In the present study it was observed that 178 women reporting previous history of RTI treatment, 70.2% (n=125/178) sought treatment from private practitioners. The similar trends of women preferring treatment for RTIs from private practitioners are reported across nations.[57,123,160] Indian women are also reported to seek treatment for RTIs from unqualified professionals viz pharmacist, primary health workers, traditional healers etc.[161] The reasons attributed for seeking treatment from alternative medicine practitioners are primarily lack of access to health care center especially in rural areas of India, the comfort levels of communication with the lady doctor who may not be available in the STI clinics run by the government and the stigma associated with these STI clinics.[162] The involvement of the above-mentioned health care providers in the STI/RTIs screening and treatment has its own benefits

and challenges reported. The wider access and confidential services provided are the major advantages reported, while improper training to diagnose and treat RTIs, ineffective partner notification due to inadequate time spend in counselling, unnecessary diagnostic test conducted leading to increase cost factor for the treatment are the major limitation.[57]

The present study demonstrated poor usage of barrier contraception among Indian women with poor partner notification system. This can contribute to re-infections of RTIs among women over a period of time.

3. Distribution of RTIs reported in the study

The two common symptoms pertaining to RTIs reported in the current study was white discharge followed by lower abdominal pain (Table 6.3) which is consistent with other studies.[72,127,128,159,163,164] The burden of RTIs is expected to be high among the middle- and low-income countries.[58] Due to ignorance, the woman is unaware of signs and symptoms and the screening facilities for RTIs present in her geographical area. The studies that were conducted to assess the awareness of RTI among Indian women, the author reported that the majority of women failed to distinguish between the physiological and pathological white discharge[160] and to understand the severity of RTI related complaint.[165] In the present study 19.7% of women in the case arm were asymptomatic at enrollment (Table- 6.4).

Among the women with clinical cervicitis (case arm) the prevalence of atleast one RTI was 94%, BV and Candida were reported in 37.4% and 5.9% women respectively (Table 6.5). The positivity rate of laboratory diagnosed RTIs in the present study was higher than that reported by Chauhan et al in their study. The author reported the positivity rates of laboratory diagnosis RTIs to be 12% and BV to be 18% among women with clinical cervicitis.[130] The prevalence of laboratory diagnosed RTIs and BV in the current study was higher due to the study design.

The most common multiple infections in the current study was BV with Candidiasis which is similar to the findings observed by Ray et al.[59] BV is the most common RTI reported in various health care settings across India.[166] This infection needs to be addressed as it has the potential to cause maternal morbidity due to its association with common conditions like PID, Chorioamniotis and Preterm labour in women.[166,167] In the present study the prevalence of BV was in par with the prevalence rates reported among other Indian studies.[59,72,125-133]

Chlamydial infection is one of the main causes for cervicitis among women globally.[56,57] It is difficult to estimate the prevalence of chlamydial infections among the general population using gold standard test (PCR/NAATS) in developing countries including India due to cost constraints.[153] In the absence of availability of affordable gold standard tests in resource constrained countries and the prompt treatment needed for this infection to prevent complications like PID and its increased morbidity, CDC recommends NGI as a surrogate marker for Chlamydial infections among women.[154] The present study demonstrates 44.1% prevalence rate of NGI among women with clinically evident infection. A community study conducted by Bote et al in urban slums of Mumbai to estimate the prevalence of lab diagnosed RTIs, the author reported 36.3% prevalence of NGI using a criteria of >30 PMNs/HPF on Gram stain smears.[125] The limitation of the study was, the author had not commented on clinical findings of the women as there can be commensal leukocytes present in vaginal flora and in the absence of clinically evident infection, there is no significance in reporting leucocytes.[150,168] The high leucocytes may also be due to recent deposition of sperms. The present study used clinical and Gram stain criteria to report NGI which is as per the recommended CDC guidelines.[154,156]

There is a huge burden of asymptomatic BV reported worldwide.[166] The prevalence of asymptomatic BV reported in our study among case arm was 19.7% while the prevalence of asymptomatic BV was observed low (4%) among women in the control arm. This is due to the

criteria of women enrolled in the control arm (asymptomatic with no clinical cervicitis) in the present study. This finding is comparable to the study conducted by Ray et al who reported the prevalence of asymptomatic BV to be 2.8% among women with no clinical signs of infection.[59] The most common RTIs reported in the present study was NGI followed by BV. No Gonococcal infection was reported among women in the present study. The limitation of the study was that we could not demonstrate the burden of T. vaginalis infection among women in case arm due to logistic issues.

4. Prevalence of epithelial cell abnormalities and RTIs reported on Pap cytology.

The Pap cytology primarily is a screening test for cervical cancer however reporting of RTIs forms a part of evaluation as per the framework provided by Bethesda system for evaluation of Pap smears. On Pap cytology the causative agents of RTIs viz BV, Candida, T.Vaginalis and Actinomyces can be determined.[169] The Pap cytology in the present study was done as a part of OPD protocol (Table 6.6). The present study demonstrated the epithelial cell abnormalities among women in the case arm (18.1%) to be significantly higher than the control arm (4.7%), demonstrating the need of promptly treating cervicitis, which can serve as a potential co-factor for persistence of HPV infection leading to precancerous and cancerous lesions of the cervix.[13,14,108] Among the RTIs, the role of Pap smear to diagnose BV has conflicting results reported from several developed and developing countries.[170,171-175] Since BV is caused by overgrowth of anaerobes which forms a part of normal vaginal flora,[150] the recommended gold standard test to diagnose BV is Nugent score. The Nugent score uses a system of scoring points allotted to different bacterial morphology, number of different bacteria present in the sample and number of lactobacilli present in the sample differentiating normal vaginal flora from BV infection.[151,152]

Using Nugent score as gold standard test, the agreement between Pap cytology and Gram stain to diagnose BV was 81.1% in case arm while the agreement was reported only in 11% of women in the control arm,(using data of Table 6.5,6.6) demonstrating that Pap cytology can be an fairly reliable tool for dual screening for BV and epithelial cell abnormalities among women with genital infections (cervicitis/cervico-vaginits).The reliability of Pap smear in diagnosing Candidiasis needs further studies, since Candida is a commensal of normal vaginal flora.[150] At present the WHO guidelines recommend the diagnosis of Candidiasis with a combination of clinical signs and microscopy of Gram stain smears.[153]

5. The Prevalence of HPV infection and histopathology results at baseline visit.

The prevalence of HPV was reported to be 14.2% in the case arm as compared to 5.1% in the control arm at baseline visit (Table 6.7). To our knowledge there is limited literature from India about the prevalence of HPV among cervicitis which is discussed in later section. As a part of SOP of the department all women enrolled in the study underwent baseline colposcopy. The VIA positivity rates in case arm was 22% (56/254) and among these women 14.3% (8/56) already demonstrated high grade precancerous lesions (CIN2 & CIN3) on histopathology (Table-6.8). The above findings reconfirm the need for screening and treating the RTIs promptly in an attempt to reduce the burden of cervical cancer.

6. Efficacy of Syndromic treatment reported in the study

Gonorrhoea, Chlamydia, Trichomonas, Bacterial vaginosis and Candidiasis are recognized causes for cervicitis/cervicovaginitis worldwide. In resource constrained countries it may not be possible to set up lab facility at every primary health care level and to introduce laboratory

tests like PCR/cell culture/ direct fluorescence assay (DFA) which need expertise to diagnose pathological agents associated with the above mentioned cervico-vaginal infections.

The WHO recommends syndromic management for resource constrained countries to effectively treat the high burden of STI/RTIs. It works on algorithms that recognize the group of symptoms which are usually caused by common pathogens and treated with an effective and evaluated drug regimens,[176-178] which are pre-packed ready to dispense kits. Most of the studies[179-183] which evaluated the algorithms of utility of syndromic case management in women are cross-sectional and restricted to demonstrating the etiological prevalence of STI/RTIs agents which again can vary across the regions and the population screened (high risk population/population-based studies/hospital-based studies). The sensitivity and specificities of these syndromic case management algorithms also depends on microbiological tests used to diagnose the pathological agents.

There are few studies that assessed the therapeutic cure rates of lab diagnosed RTIs of syndromic treatment at follow up visit from India. The study conducted in India by Parmar et al reported the overall therapeutic cure rates of lab diagnosed RTIs to reduce from 26% at baseline to 9.6% at follow up visit at an interval of seven days.[72] The other study conducted by Kore et al,[184] to assess the efficacy of one day regime of syndromic treatment among 100 women with symptoms pertaining to lower genital tract infection attending the Gynae OPD, the author reported the symptomatic cure rates for white discharge to be 88%, urinary symptoms to be 91% and pruritis to be 62% at a follow up interval of 14 days. The limitation of this study was no laboratory tests were conducted to avoid subjective biases.

In the present study the syndromic treatment compliance was 96.7% and 94.7% for partner treatment among women in the case arm at followed up visit. The compliance for use of barrier contraception/sexual abstinence reported in the case arm was 94.7% and 80.9% in control arm

at followed up (Table 6.9). We could demonstrate significant microbiological cure rates for each etiological pathogen for cervicitis/cervicovaginitis among women in the case arm who received syndromic treatment as per guidelines[56,156] at baseline visit. The overall cure rates in the case arm was 91.4% at a median follow up period of 13 days. The burden of BV in the case arm was reduced from 43.8% to 3.7%, NGI reduced from 45% to 4.1% and Candidiasis from 12.3% to 0.8%. Among the women in the control arm who received no treatment at baseline visit, the burden of asymptomatic BV increased from 3.7% to 6.6% at median follow up interval of 11 days (Table 6.10).

The study supports the syndromic treatment recommended by WHO in resource constrained country like India, after a per speculum examination, where laboratory diagnosis is not available. In the present study women in the case arm received syndromic treatment to reduce the inflammation and mucopurulent discharge associated with cervicitis since the objective of the study was to determine the effect of mucopurulent cervicitis on HPV detection by HC2 test before and after treatment of mucopurulent cervicitis.

7. HPVDNA HC2 test results among women at follow up visit

In case arm we expected the detection rates of HPV by HC2 test to improve after treating mucopurulent cervicitis (caused by RTIs) with antibiotics if these women were harboring HPV infection at baseline visit that was initially missed by HC2 test. The control arm would reflect the natural history of HPV and the above-mentioned changes were not expected in control arm. In the present study there were conversion of HPV test results demonstrated in both the study arms in terms of repeat test positive/negative and detection of HPV at follow up visit among initially HPV negative women at a short interval of two weeks (Table 6.11). Below are the various reasons proposed for these findings.

7.a. Repeat test positive (Persistence) for HPVDNA on follow up visit- probable causes

The outcome of HPV test on follow up visit (follow up range 7-30 days, median follow up case arm-13 days, control arm-11 days) with that of baseline results, demonstrates some key findings of the study with respect to presence of laboratory reported RTI infections (Fig 6.3). The oncogenic HPV itself is not associated with inflammation of the cervix. It is assumed that the cervicitis caused by exogenous (Neisseria Gonorrhoea, Chlamydia, Trichomonas) or endogenous organisms (Bacterial Vaginosis, Candidiasis) results in change of microbiological flora of vagina leading to decrease local immunity in terms of decreased lactobacilli count, which in turn favor the acquisition of HPV which is the main cause of cervical cancer. Inflammation of cervix associated with RTIs causes break in the cervical epithelium helping the HPV to gain entry into the actively proliferating basal cells of cervical epithelium.[108] Inflammation is also known to cause DNA damage of the host cell leading to integration of viral DNA, which leads to gradual progression of HPV infection to precancerous and cancerous lesions of cervix.[13]
The persistence of HPV at follow up visit was reported higher among women in the case arm (74.3%) than in the control arm (53.8%) as seen in fig 6.3, demonstrating that existing cervicovaginal co infections may interfere with the natural history of HPV infections in potentiating the infection to be persistent.[13,14,118,119]

7.b. Repeat test negative (Clearance) for HPV infection on follow up visit – probable causes

The study demonstrated clearance of HPV (HPV detected at baseline visit and not detected on follow up visit) within a short median interval of 11 and 13 days in control and case arm respectively (Fig 6.3). The proposed causes of these changes are –

7.b.1. Clearance rates depends on screening intervals of HPVDNA test.

The HPV infections are transient and mostly clear within a short period of time without causing any abnormalities on cervical smear or colposcopy.[11,86,185] Different authors reported different clearance rates at various intervals of time.[11,86,185-189] At present there is no consensus on definition of transient period (clearance) of HPV infection (Annexure 1.Table 3).

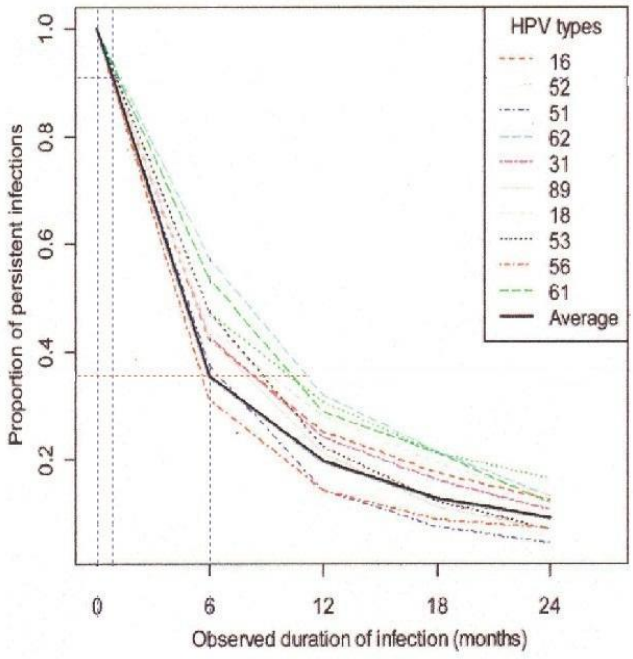
A study that looked into clearance of HPV infection at a short interval of 7 days among women in the age group of 18-35 years, the author reported 33.7% clearance rate of oncogenic HPV infection.[190] The author reported high fluctuation of genital HPV in short interval of time. The author proposed these fluctuations of genital HPV to host differences (innate immunity), error in sampling technique, gaps in understanding patterns of HPV multiplication and limitations of test assay in terms of viral load below the detection threshold of the HC2 test.

Another retrospective study which looked at the clearance rates of HPV at a shorter interval of 3 months in 599 women with oncogenic HPV type enrolled into a population-based cohort study (Guanacaste, Costa Rica), reported a clearance rate of 34% among women who were in the age group of 30 and above. The author commented that the clearance of HPV would be actually faster than we could measure and the clearance estimates are affected by the screening interval.[185]

An RCT conducted by Basu et al to evaluate an herbal vaginal cream for clearance of HPV type 16 and 18 infection among women aged 30-60 years reported a clearance rate of 73.3% at a median interval of 1 month among women enrolled in the control arm (no treatment given).[191]

The findings of ALTS trial (fig below) demonstrated average HPV clearance rate of 64% at an interval of 6 months. Moreover, there was a steep slope of clearance observed up to 6 months. The study demonstrates around 15% clearance of HPV at 1 month.[86] Schiffman et al in their

review article mentioned the clearance rates of HPV infection to be rapid within the first few months after infection and decreases over a period of time. The reasons for rapid clearance of large fraction of HPV infection still remains unknown.[11,86]



HPV clearance rates reported in ALTS trial (Atypical squamous cells of undetermined significance/Low grade squamous intraepithelial lesion triage study)

7.b.2. Clearance depends on Innate host immunity

It is known that the lifetime risk of a sexually active women to acquire HPV infection is 80-90%, but majority of women clear the infection without being clinically detected. The HPV infections are transient and do not induce inflammatory reaction and the virus being intracellular gets shed from epithelial layer of cervical epithelium without being exposed to lymph nodes where antibodies responses are initiated. This supports innate immunity (dendritic cells, interferons, cytokines, neutrophils, macrophages) to play a major role as a first line defense to clear the HPV infection.[88,192,193] The failure to mount an effective innate

immunity or cell mediated immunity to clear or control the infection leads to persistence of HPV virus increasing the probability of precancerous or cancerous lesions.

7.b.3**.** Clearance rates are affected by type specific HPV

The population-based cohort of Bogota (Colombia) of 227 women aged 13-85 years (median age 29) with normal cytology and HPV positive test at baseline were monitored for a mean follow-up period of 5.3 years for the type specific clearance rates of HPV at 6 months,1 year and 5-year interval. The author reported overall rapid clearance rates for low risk HPV types than the oncogenic HPV types. Among the oncogenic HPV types, HPV type 18 had a faster clearance rate than type 16.[188]

The Hawaii cohort study[186] enrolling 972 sexually active women in the age group of 18-85 years (median age 33 years) were monitored for acquisition, clearance and persistent of oncogenic and low risk HPV types for a mean duration of 15 months. The author commented the clearance to be affected by viral type. Types 16, 18 and 33 demonstrated lowest clearance rates among the oncogenic HPV types.

The findings of the Population-Based Screening Amsterdam (POBASCAM) trial, a prospective RCT,[194] which assessed the clearance rates of 14 oncogenic HPV type among women with and without abnormal cytology, aged 30- 60 years, demonstrated an overall decreased clearance for type specific HPVs 16,18,31 and 33 compared to other oncogenic types at 6 months. The study demonstrated statistically significant decreased clearance rates for HPV type 16 irrespective of cytological test results.[68]

In the above-mentioned studies, oncogenic HPV types demonstrated a longer clearance time than low risk virus. Type 16 was uniformly reported to be a common HPV type with the delayed clearance rates. The clearance also depends on infection with single/multiple oncogenic types

and the time interval between the two-tests. Since the present study used HC2, a qualitative test, the test comments only on being positive or negative for 13 high risk HPV type without commenting on the HPV genotypes in the cervical sample. In view of the above limitation with HC2 test, it was difficult to estimate which HPV type was women infected with, at baseline visit.

7.b.4 -There lies a possibility of HPV infections to be transient due to recent deposition of semen through sexual contact with an infected partner. These infections are likely to disappear if the test is repeated within a couple of weeks. In the present study, the history of sexual activity at baseline visit was not captured.

7.b.5. Role of Viral loads of HPV in clearance

It was observed in the present study that the women who cleared the infection on follow up visit (a median follow up of 13 days in case arm and 11 days in control arm) majority of them had RLU titers below 10 at baseline visit (Table 6.12). No women who cleared the infection at follow up visit demonstrated titers above 100 at baseline visit. These findings demonstrate that, the RLU titers load of HPV infection may be one of the determinants of HPV clearance observed in the present study at a short interval of time. At present there is conflicting role of RLU titers in determining persistence / clearance of HPV infection[108,187,188,195] though the findings of the present study are similar to findings reported by Kim et al.[195] Woodman et al[108] in their review article mentioned gaps in understanding the relation between viral load, type specific oncogenic HPV and cervical cancer. The author mentions, association between the increasing viral load and cervical cancer lesion has been demonstrated for oncogenic type 16 but not for type 18. There also lies a possibility of integration of HPV virus into the host cell leading to low viral load captured by molecular test in case of cervical cancerous lesion.

7.b.6. Issue of Cross reactivity of HC2 test probes leading to false positive test results

The HC2 test has a limitation of cross-reactivity of probes reported leading to false positive test results.[92,93,143,196,197] The oncogenic and the low risk HPV types share Alpha clades due to their similar nucleotide sequence. This forms the basis of cross-reactivity associated with HC2 test. The cross reactivity is defined as the samples tested positive on HC2 test and demonstrating low risk genotypes on PCR test or a negative PCR test. As per Qiagen HC2 manufacturer, the cross reactivity could be demonstrated for low risk HPV types 6,11,42,53,54,55,56.[143] The cross-reactivity of the probes results in weak RLU titers. At present there is no consensus on specific cut off RLU titer values to address the issue of cross reactivity of probes. There are no studies to demonstrate if cross reactivity of probes in HC2 test would occur with bacteria or bacterial plasmids which may be commensal in female anogenital tract or present during genital infection, though the manufacturer claims no such occurrence.[143] In the present study, women in the case arm who showed clearance of HPV on follow up visit, 55.6% were with weak RLU titers of less than two at baseline. In view of above findings the possibility of cross reactivity of HC2 test probes leading to low RLU titers associated with weak signals cannot be entirely ruled out to be the causative factor for false positive test results at baseline visit.

Majority of the studies conducted to measure clearance / persistence rates of cervical HPV reported clearance rates at longer intervals of time, mostly at 6 months / 12 months / 18 months which may not reveal the true dynamic picture of HPV infections within the time period of two tests. The above-mentioned factors like immunity, screening intervals, and type specific genotype has the potential to play a key role in the clearance rates of cervical HPV infections. HPV infections are transient so repeating HPVDNA test at a shorter interval of time has no added advantage and may not be cost effective for cervical cancer screening. In the present

study the HC2 test was repeated at a short interval (7-30 days) of time to address the false negativity issue of the HC2 test due to co-infection with RTIs associated with clinical cervicitis.

7.c. HPV not detected at baseline & detected at follow up visit- probable cause

In the present study 5.3 % women were detected with HPV infection, who were tested negative at baseline visit in the case arm at median follow up interval of 13 days. These women had received treatment for mucopurulent cervicitis demonstrating an overall therapeutic cure rate of 91.4%. This supports the hypothesis of the current study that mucopurulent discharge associated with RTIs can affect the cellularity for HC2 test. However, the control arm in the study also demonstrated 1.7 % increase in detection rates of HPV on follow up visit at median follow up interval of 11 days which was not expected (Fig 6.3). The proposed causes for these (negative at baseline and positive on follow up) variations are-

7.c.1- Limitation of HC2 assay

Since HC2 test has no inbuilt system to monitor cell adequacy, probably less the cell pick up leading to less viral copies below a detectable threshold of HC2 test may affect the test results (false negative), which is our proposed hypothesis.[75,190,198] It was also observed that the women detected with HPV infection at follow up visit who had negative HPV test results at baseline visit demonstrated low RLU titers between 1-10 at follow up visit (Table 6.12).

7.c.2- Gaps in understanding HPV latency and differentiating new HPV infection.

Majority of cervical HPV infections being transient is mainly cleared due to innate immunity. HPV gets shed from superficial cervical epithelial layer without initiating cell mediated/ humoral immune responses,[193] hence women can be infected again with the same or other HPV type(s) in future depending on her sexual activities.[199] Moreover, among the risk determinants

of HPV infections the most important determinant, consistently reported in epidemiological studies is sexual activity.[200] At present there is a gap in the literature in differentiating a new HPV infection with reactivation of latent HPV. The only study powered to understand the above-mentioned difference was by Trottier et al,[201] who monitored a cohort of Brazilian women, aged 18-65 years for 10 years at an interval of 4-6 months. The author reported that women can be infected with the same type(s) of HPV or other type(s) of HPV at multiple times in her lifetime. The author mentions that the second peak of HPV infections demonstrated during the old age which is proposed to be due to decreased immunity leading to reactivation of HPV is mainly associated with sexual activity and immunity has no role in reactivation or the latency period of cervical HPV. The author demonstrated Relative Risk (RR) of 3.7 for reinfection with the same HPV type(s) and RR of 2.3 for reinfection with different HPV type(s) among women who reported prior HPV infection(s). The author reported RR of 2.8 for reinfection among women aged 40-65 years that were clearly reported due to new sexual partners. The author concluded that infection and reinfection with HPV type(s) are strongly associated with sexual activity.

7.c.3. Controversial role of barrier contraception (condoms) in preventing HPV infections

At present the role of barrier contraception to completely prevent HPV infections has conflicting results.[202,203] Since HPV can be transmitted through skin to skin contact, apart from sexually transmitted route, it becomes difficult to assess the role of condoms in complete prevention of HPV infection. The major issue with cross-sectional studies that evaluated the role of condoms in preventing HPV infection is the transient nature of HPV infection. Manhart LE et al,[203] reviewed the role of condoms in providing protection against HPV and genital warts, the author concluded that at present available literature was inconsistent to form any precise statement on role of condoms to prevent HPV infection. The only prospective longitudinal study[109] undertaken on 82 women in the age group of 18-22 years, which was

powered to understand the role of condoms in preventing HPV infection, concluded that women whose male partners used condoms 100% of time had 70% less chances to acquire new HPV infection than the women whose male partners used condoms. The strength of the study was, it limited the biases of recording the condom usage history using the web recorder instead of a face to face interview. The limitation of the study was the unavailability of data on close skin to skin contact which is a recognized mode of HPV transmission and condom breakage. The author commented that skin to skin contact of perineum may be responsible for HPV infections reported among women whose partner regularly used condoms.

At present, even though condoms have shown to protect STIs, there is no adequate scientific evidence to establish the role of condoms in completely preventing genital HPV infections.[204] The limitation of the unavailability of specific data in literature review on close skin to skin contact which is a recognized mode of HPV transmission and subjective biases in reporting history of condom breakage gives rise to controversial role of condoms in preventing HPV infections.

8. The known factors for false negative HC2 test results were controlled in the study

The burden of false negative test errors for HC2 test is estimated to be around 1.1% to 7.5%.[205] The common factors known to cause false negative test results of HC2 are vaginal creams and jelly,[143] improper sampling technique that may result in less cell collection,[52] temperature fluctuations during transportation and storage that may cause denaturation of HPV DNA.[48] In the current study, the illustrated factors for false negative test errors were controlled

1. Since there is a possibility of substances like antifungal cream, vaginal pessaries affecting the cell adequacy,[143] women in the case arm who were advised vaginal pessaries as a part of RTI treatment were advised to stop the use of pessaries at least 3 days prior to second sample collection.

2. The sampling technique, transportation and storage of samples were conducted as per the SOP of Microbiology department, TMH, which is NABL accredited.

3. To prevent new incident HPV infections during study period all women were counselled for sexual abstinence or use of barrier contraception.

4. In the present study, the follow up visit was planned at interval of two weeks to prevent the biases of new incident infection among women enrolled in the study. At present there is not much quality evidence on latency period of cervical HPV. There is a gap in understanding the time taken by the infected basal / parabasal cervical cells to reach superficial epithelium from where these cells are exfoliated and can be detectable by HPVDNA tests. The cervical HPV infection is reported to have a latency period ranging from 2–4 weeks to months or years and the duration of this phase of latency probably is also related to the load of virus.[89,107] The shortest interval reported by Rachel LW et al between the penetrative sex and incident HPV detection was 20 days in a prospective study.[109] In our study, accounting for the minimal period of latency of 2 weeks, all the women were advised to follow up within 14 days.

9. The impact of co-infections of RTIs on HPV detection by HC2 test. (Primary objective)

In the present case-control study there was no difference among women enrolled in the case and the control arms pertaining to sociodemographic and reproductive characteristics (Table 6.1) except for clinical cervicitis in the case arm at baseline visit. The prevalence of lab diagnosed RTIs and HPV were reported to be 94% (Table 6.5) and 14.2% (Table 6.7) respectively in the case arm at baseline visit. All women in the case arm received syndromic treatment at baseline visit.

At follow up visit, the compliance to sexual abstinence/ barrier contraception use was reported in 94.7% and 80.9% among women in the case and the control arms respectively (Table 6.9). The burden of RTIs in the case arm demonstrated reduction from 94% at baseline to 8.6% on follow up visit after syndromic treatment among the women who followed up (Table 6.10). All

the known factors for false negative test results of HC2 test were controlled in the study as mentioned earlier.

As shown in Table 6.13 the overall persistence of HPV was 10.7% in the case arm versus 2.9% in the control arm at follow up visit. The study demonstrated overall 4.5% increase in detection rates of HPV by HC2 test after treating cervicitis at a median follow up visit of 13 days. This finding supports our hypothesis that the mucopurulent cervicitis can influence the results of HC2 test. However, the present study also demonstrated overall 1.6% increase in the positivity rates of HPV among women in the control arm, which was not expected after controlling factors for the known false negative test results.

There were conversions of HPV outcome status (HPV detected / HPV not detected) demonstrated within and between the study arms (case/control) at different time points (Fig 6.3). There were fixed variables like clinical cervicitis (fix variable) which the present study was powered to study its effect on HPV outcome status (outcome variable) after intervention at follow up visit (fixed variables). Since the conversion of HPV outcome status were demonstrated in both the study arms, we assume there would be random factors/events as mentioned below which may have influenced HPV test results leading to these variations which needs to be accounted -

1.The possibility of women wrongly reporting the history of condom usage.

2.Reporting of sexual activity may be subject to cultural biases especially among Indian women.

3. Clearance due to low RLU titers.

4. Role of innate host immunity in clearance of cervical HPV.

5. Gaps in understanding HPV latency.

6. Many more unknown factors that may be present due to gaps in literature pertaining to the natural history of HPV.

Amidst above variations in HPV outcome status observed in the study which are assumed to be caused due by uncontrolled factors (reporting of sexual history / condom history by women) and unassessed biological host factors (Innate immunity, other unknown factors), it becomes important to know the true effect of our intervention in improving the detection rates of HPV by HC2 test after treating cervicitis in the case arm (proposed hypothesis of the study). The generalized Linear Mixed Effect (LME) model was used to analyze primary objective of the study. The model took into consideration the fixed variables (clinical cervicitis & visits) which the study was powered for and also the individual level subjective variations caused by the above-mentioned random factors/events by including individual woman as random variable in the LME model. The model demonstrated a significant role of clinical cervicitis (mucopurulent discharge) to influence the detection rates of HPV by HC2 test (Table 6.14).

10. Prevalence of HPV in clinical cervicitis and lab diagnosed RTIs. (secondary objective)

The present study demonstrated, the positivity rate of HPV to be significantly higher in women with cervicitis (14.2%) versus (5.1%) women without cervicitis. The risk was observed to be three times higher among women with cervicitis (Table 6.15)**.** The studies conducted in China, Costa-Rica, Brazil, and Turkey have shown the prevalence of HPV among women with cervicitis / Mucopurulent cervicitis to be in the range of 5%-58.7%.[64-66,135,136] These studies varied in the study design and the method of diagnosing cervicitis either clinically or on Pap smear. The clinical cervicitis can have subjective biases, while Pap smear is not a recognized standard test to diagnose cervicitis. The present study used clinical diagnosis supported by lab diagnosis (Gram stain) to establish diagnosis of cervicitis. As discussed earlier, cervicitis caused by exogenous or endogenous organisms result in the acquisition of HPV and helps in integrating the HPV in host DNA, which can modulate oncogenic HPV infection to precancerous and cancerous lesion.[13,108]

There is a strong emerging evidence for association of BV and Chlamydial infection with uterine cervical oncogenic HPV infection.[67-71,119] The risk of squamous cell carcinoma has been reported to increase among women with co-infection of HPV and Chlamydia.[68] At present, among the other RTIs, the association of persistent HPV with trichomonas infection and Candida is controversial and needs further studies.[138-140] In the present study, among the women with lab diagnosed RTIs the prevalence of co-infection of HPV with BV, multiple infections (BV with Candidiasis) and NGI was 13.3%, 11.8% and 13.4% respectively (Table 6.16). To our knowledge there is limited data of prevalence of co-infection of RTIs with HPV reported in general population of India. The only study conducted by Basu et al,[142] in 45 female sex workers to estimate the burden of co-infection of HPV with STIs, the author reported a prevalence of 88.6% for candidiasis, 22.9% for Trichomoniasis and 14.3% for Chlamydial infection among women with HPV infection. The limitation of study was small sample size and the findings cannot be generalized. The present study demonstrated high prevalence of HPV among women with cervicitis and high burden of coinfections with RTIs needing screening of women for HPV and RTIs.

Limitations of the present study.

1. The possibility of cross reactivity of HC2 test probes with low RLU titers resulting in false positive HPV results at baseline visit cannot be entirely ruled out. At present there is no consensus on the cut off ratios for cross-reactivity of HC2 test probes and role of RLU titers in the persistence or clearance of HPV infections.

2. The results of HC2 test were not validated by PCR test for cell adequacy due to cost constraints.

3. Due to cost constraints, we could not apply gold standard tests to diagnose gonococcal and Chlamydial infections, though our study would represent the true scenario where a simple Gram stain test is not routinely done in every Indian health care setting. PCR and NAATs are the recommended tests for Gonococcal and Chlamydial infection which cannot be cost-effective tools for screening in developing countries. Since these organisms are associated with high morbidity and mortality among women needing prompt screening and treatment the present study used the lab diagnostics recommended by CDC.

4. The present is a case-control study, with a good quality. The study forms the first evidence for potential influence of cervicitis on HC2 test results. A robust analysis was used for the primary objective of the study.

CHAPTER 8

SUMMARY AND CONCLUSION

CHAPTER 8

SUMMARY AND CONCLUSION

The developed countries are shifting from cytology-based screening to molecular based screening for cervical cancer, the advantage being, high sensitivity and longer screening intervals needed due to the high negative predictive value of the molecular test (HC2). There are several advantages of setting up of HC2 machines in countries; viz. they can run high volumes of HPVDNA tests at a time, the requirement of skilled laboratory technicians would reduce and the reporting would be standardized. At present there are concerns regarding low sensitivity of HC2 test reported from developing countries including India. With the introduction of care HPV test, that can be conducted at the field site and is supposed to be low cost, it becomes important to address the issue of low sensitivity of the HC2 test from Indian perspective. The present study, to our knowledge, is one of the first studies, statistically powered to evaluate the effect of co-infection with RTIs on the test results of HC2. The study also demonstrated natural history of HPV among women with and without cervicitis over a shortest median period of 2 weeks. Below mentioned are some key findings of the study.

Key findings (Conclusions)

1. The point prevalence of STIs/RTIs in India is estimated to be around 30 million. The burden of HPV co-infections with STI/RTIs is also expected to be high among Indian women. There is not enough literature available for the above-mentioned burden of infections from Indian context. In the present study the prevalence of HPV and RTIs among women with mucopurulent cervicitis (clinical cervicitis) was 14.2% and 94.3% respectively at baseline visit. The overall burden of HPV co-infection with RTIs

demonstrated in the present study was 12.5% at baseline. The most common RTIs reported were NGI and BV.

2. The study demonstrated good efficacy of syndromic management for RTIs in significantly reducing the burden of laboratory confirmed RTIs. The study findings support syndromic treatment for RTIs in resource constrained countries after a per speculum examination. Majority of symptomatic women (70%) seek treatment for RTI related complaints from private health practitioners in India. The above findings emphasise the need of sensitizing these health care providers in syndromic management to assure quality treatment for RTIs. Poor partner notification was reported by women who had received previous treatment for RTIs in the present study. This can lead to reinfections and incomplete treatment. Strong partner notification system will ensure comprehensive treatment for RTIs.

3. The prevalence rates of HPV infection by HC2 test among women with cervicitis was statistically higher (14.2%) than women in the control arm (5.1%) at baseline. At follow up visit the persistence rates of HPV among women with cervicitis was higher (10.7%) than women without cervicitis (2.9%), as demonstrated by repeat HPV test. These findings illustrate the increased risk of persistence of HPV among women with RTI co-infections. We however do not rule out the possibility of other hitherto unknown variables, which could be the subject of future investigation.

4. The epithelial cell abnormalities were reported to be significantly higher among women with cervicitis (18%) as compared to women without cervicitis (4.8%), reinforcing the role of inflammation and co-infection in modulating the progression of HPV infection to precancerous/ cancerous lesion of cervix. The above findings demonstrate the

importance for prompt screening and treatment of RTIs in an attempt to reduce the cervical cancer.

5. The overall prevalence of BV in case arm was 37.4% and 6.8% with other pathogens. This infection needs prompt screening and treatment due to increased morbidity associated with it. Using Nugent score on Gram stain as gold standard test, the agreement between Pap cytology and Gram stain to diagnose BV was reported to be 81.1% among women with cervicitis. This demonstrates that Pap cytology can be used in dual screening for BV and cervical cancer which are the common cause of morbidity and mortality among Indian women. This eliminates the requirement of additional diagnostic tests like the Gram stain for diagnosing BV, which itself is not routinely done in Indian health-care settings.

6. The present study demonstrated the overall detection rates of HPV by HC2 test to improve by 4.5% among women treated for mucopurulent cervicitis. On controlling the biological and behaviour determinants by modelling, the study demonstrated influencing role of mucopurulent discharge associated with cervicitis on the test results of HC2.

   The outcome of the present study provides proof of concept that concomitant RTIs interfere with the accuracy of HPV detection by HC2 method, which was our proposed hypothesis.

CHAPTER 9

REFERENCES

References:

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

3. Gakidou E, Nordhagen S, Obermeyer Z. Coverage of cervical cancer screening in 57 countries: low average levels and large inequalities. PLoS Med. 2008;5(6):e132.

4. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer. 2017;141(4):664-70.

5. Mishra GA, Pimple SA, Shastri SS. Prevention of cervix cancer in India. Oncology. 2016;91(Suppl.1):1-7.

6. Sankaranarayanan R, Swaminathan R, Brenner H, Chen K, Chia KS, Chen JG, et al. Cancer survival in Africa, Asia, and Central America: a population-based study. Lancet Oncol. 2010;11(2):165-73.

7. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12-9.

8. Munoz N, Bosch FX, De Sanjose S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348(6):518-27.

9. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. Infect Agent Cancer. 2009;4(1):8.

10. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Meeting, World Health Organization, International Agency for Research on Cancer. Human papillomaviruses. World Health Organ. 2007.

11. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human papillomavirus testing in the prevention of cervical cancer. J Natl Cancer Inst. 2011;103(5):368-83.

12. World Health Organisation. https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(hpv)-and-cervical-cancer downloaded on 12.6.2019.

13. Williams VM, Filippova M, Soto U, Duerksen-Hughes PJ. HPV-DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress. Future virol. 2011;6(1):45-57.

14. Castle PE, Giuliano AR. Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients—assessing their roles as human papillomavirus cofactors. JNCI Monographs. 2003;2003(31):29-34.

15. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis—role of parity, oral contraceptives, and tobacco smoking. JNCI monographs. 2003;2003(31):20-8.

16. Jensen KE, Schmiedel S, Norrild B, Frederiksen K, Iftner T, Kjaer SK. Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. Br J Cancer. 2013;108(1):234.

17. Munoz N, Méndez F, Posso H, Molano M, Van Den Brule AJ, Ronderos M, et al, Instituto Nacional de Cancerologia HPV Study Group. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis. 2004;190(12):2077-87.

18. Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus–positive women. J Natl Cancer Inst. 2005;97(8):577-86.

19. Laara E, Day N, Hakama M. Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. Lancet. 1987;329(8544):1247-9.

20. Nygard JF, Skare GB, Thoresen SO. The cervical cancer screening programme in Norway, 1992–2000: changes in Pap smear coverage and incidence of cervical cancer. J Med Screen. 2002;9(2):86-91.

21. Lazcano-Ponce EC, Moss S, de Ruíz PA, Castro JS, Avila MH. Cervical cancer screening in developing countries: why is it ineffective? The case of Mexico. Arch Med Res. 1999;30(3):240-50.

22. Othman NH, Rebolj M. Challenges to cervical cancer screening in a developing country: The case of Malaysia. Asian Pac J Cancer Prev. 2009;10(5):747-51.

23. Aggarwal P, Batra S, Gandhi G, Zutshi V. Comparison of Papanicolaou test with visual detection tests in screening for cervical cancer and developing the optimal strategy for low resource settings. International Journal of Gynecologic Cancer. 2010;20(5):862-8.

24. Shastri SS, Dinshaw K, Amin G, Goswami S, Patil S, Chinoy R, et al. Concurrent evaluation of visual, cytological and HPV testing as screening methods for the early detection of cervical neoplasia in Mumbai, India. Bull World Health Organ. 2005;83:186-94.

25. Shastri SS, Mittra I, Mishra GA, Gupta S, Dikshit R, Singh S, et al. Effect of VIA screening by primary health workers: randomized controlled study in Mumbai, India. J Natl Cancer Inst. 2014;106(3):dju009.

26. Sankaranarayanan R, Wesley R, Thara S, Dhakad N, Chandralekha B, Sebastian P, et al. Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. Int J Cancer. 2003;106(3):404-8.

27. 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 Gynecol Surv. 2003;58(8):543-50.

28. Sankaranarayanan R, Rajkumar R, Esmy PO, Fayette JM, Shanthakumary S, Frappart L, et al. Effectiveness, safety and acceptability of 'see and treat' with cryotherapy by nurses in a cervical screening study in India. Br J Cancer. 2007;96(5):738.

29. World Health Organization (WHO). Comprehensive Cervical Cancer Control. A guide to essential practice. Geneva; WHO 2006.

30. World Health Organization, Programme on Cancer Control Department of Reproductive health. Cervical cancer screening in developing countries: report of a WHO consultation. Geneva;World Health Organization:2002.

31. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009;360(14):1385-94.

32. Salmeron J, Lazcano-Ponce E, Lorincz A, Hernández M, Hernández P, Leyva A,et al. Comparison of HPV-based assays with Papanicolaou smears for cervical cancer screening in Morelos State, Mexico. Cancer Causes & Control. 2003;14(6):505-12.

33. Flores Y, Bishai D, Lazcano E, Shah K, Lbrincz A, Hernández M, et al. Improving cervical cancer screening in Mexico: results from the Morelos HPV Study. Salud Publica Mex. 2003;45(S3):388-99.

34. Markowitz LE, Tsu V, Deeks SL, Cubie H, Wang SA, Vicari AS, et al. Human papillomavirus vaccine introduction–the first five years. Vaccine. 2012;30:F139-48.

35. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Clinical applications of HPV testing: a summary of meta-analyses. Vaccine. 2006;24:S78-89.

36. Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, et al. European guidelines for quality assurance in cervical cancer screening. Second edition —summary document. Ann Oncol. 2010;21(3):448-58.

37. Australian Government Department of Health. National cervical screening program.www.cancerscreening.gov.au/internet/screening/publishing.nsf/Content/cervical-screening-1 downloaded on 12.8.2019.

38. https://cervicalcancernews.com/2015/11/17/netherlands-start-first-hpv-primary-screening-program/ downloaded on 12.8.2019

39. Inoue M, Sakaguchi J, Sasagawa T, Tango M. The evaluation of human papillomavirus DNA testing in primary screening for cervical lesions in a large Japanese population. International Journal of Gynecologic Cancer. 2006;16(3):1007-13.

40. Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet. 2003;362(9399):1871-6.

41. Petry KU, Menton S, Menton M, van Loenen-Frosch F, de Carvalho Gomes H, Holz B, et al. Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. Br J Cancer. 2003;88(10):1570.

42. Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med. 2007;357(16):1579-88.

43. Bigras G, De Marval F. The probability for a Pap test to be abnormal is directly proportional to HPV viral load: results from a Swiss study comparing HPV testing and liquid-based cytology to detect cervical cancer precursors in 13842 women. Br J Cancer. 2005;93(5):575-81.

44. Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M, et al. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. Br J Cancer. 2001;84(12):1616.

45. Coste J, Cochand-Priollet B, de Cremoux P, Le Galès C, Isabelle C, Vincent M, et al. Cross sectional study of conventional cervical smear, monolayer cytology, and human papillomavirus DNA testing for cervical cancer screening. BMJ. 2003;326(7392):733.

46. Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. J Natl Cancer Inst. 2006;98(11):765-74.

47. Kitchener HC, Almonte M, Thomson C, Wheeler P, Sargent A, Stoykova B, et al. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol. 2009;10(7):672-82.

48. Almonte M, Ferreccio C, Winkler JL, Cuzick J, Tsu V, Robles S, et al Cervical screening by visual inspection, HPV testing, liquid-based and conventional cytology in Amazonian Peru. Int J Cancer. 2007;121(4):796-802.

49. Blumenthal P, Gaffikin L, Chirenje ZM, McGrath J, Womack S, Shah K. Adjunctive testing for cervical cancer in low resource settings with visual inspection, HPV, and the Pap smear. Int J Gynaecol Obstet. 2001;72(1):47-53.

50. Kuhn L, Denny L, Pollack A, Lorincz A, Richart RM, Wright TC. Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. J Natl Cancer Inst. 2000;92(10):818-25.

51. Sarian LO, Derchain SF, Naud P, Roteli-Martins C, Longatto-Filho A, Tatti S, et al. Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America: This report refers to partial results from the LAMS (Latin American Screening) study. J Med Screen. 2005;12(3):142-9.

52. Sankaranarayanan R, Chatterji R, Shastri SS, Wesley RS, Basu P, Mahe C, et al. Accuracy of human papillomavirus testing in primary screening of cervical neoplasia: results from a multicenter study in India. Int J Cancer. 2004;112(2):341-7.

53. Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. JAMA. 2000;283(1):87-93.

54. Supplements to the European Guidelines on Prevention of Cervical Cancer. M. Arbyn. LancetOncol 2009. https://www.seap.es/c/document_library/get_file?uuid=0b3b449e-9f70-4cc6-9170-91af423824b&groupId=10157.downloaded on 17/8/2015.

55. Bruni L, Diaz M, Castellsagué M, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. Journal of Infectious Diseases. 2010;202(12):1789-99.

56. National guidelines on prevention, management and control of reproductive tract infections and sexually transmitted infections available on http://indiahivinfo.naco.gov.in/naco/resource/nationalguidelines- prevention-management-and-control-reproductive-tract-infections-and. downloaded on 17-08-2015

57. Center for Disease Control. Sexually transmitted infections in developing countries. Current concepts and strategies on improving STI prevention,treatment and control.

http://siteresources.worldbank.org/INTPRH/Resources/STINoteFINAL26Feb08.pdf.downloaded on 17/8/2015.

58. World Health Organisation. https://www.who.int/en/news-room/fact sheets/detail/sexually-transmitted-infections downloaded on 14.7.2019

59. Ray K, Bala M, Bhattacharya M, Muralidhar S, Kumari M, Salhan S. Prevalence of RTI/STI agents and HIV infection in symptomatic and asymptomatic women attending peripheral health set-ups in Delhi, India. Epidemiol Infect. 2008;136(10):1432-40.

60. Mayaud P, Mabey D. Approaches to the control of sexually transmitted infections in developing countries: old problems and modern challenges. Sex Transm Infect. 2004;80:174-182.

61. NACO. Report on mid-term review of sexually transmitted infection services. December 2009.http://www.naco.gov.in/upload/Publication/STI%20RTI%20services/Other%20STI%20Material/STI%20RTI%20MONOGRAPH%20_NACP-III-.pdf.Downloaded on 28/3/2015.

62. Nirmal K, Saha R, Ramachandran VG, Bhattacharya SN. PREVALENCE OF STIs AMONG ATTENDEES OF TERTIARY HEALTH FACILITIES IN NORTH INDIA: A HOSPITAL BASED STUDY PREVALENCE. Intl.J.Clin.Diag.Res. 2017;5(6):II

63. Patel V, Weiss HA, Mabey D, West B, D'souza S, Patil V,et al. The burden and determinants of reproductive tract infections in India: a population based study of women in Goa, India. Sexually transmitted infections. 2006;82(3):243-9.

64. Castle PE, Hillier SL, Rabe LK, Hildesheim A, Herrero R, Bratti MC, et al. An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). Cancer Epidemiol Biomarkers. 2001;10(10):1021-7.

65. Yuan X, Yang Y, Gu D, Liu H, Yang H, Wang M. Prevalence of human papillomavirus infection among women with and without normal cervical histology in Shandong Province, China. Arch Gynecol Obstet. 2011;283(6):1385-9.

66. Liu W, Wu EQ, Yu XH, Feng LH, Jiang CL, Zha X, et al. Detection of human papillomavirus genotypes associated with mucopurulent cervicitis and cervical cancer in Changchun, China. Int J Gynaecol Obstet. 2013;120(2):124-6.

67. Hawes SE, Kiviat NB. Are genital infections and inflammation cofactors in the pathogenesis of invasive cervical cancer? J Natl Cancer Inst. 2002;94(21):1592-3.

68. Smith JS, Munoz N, Herrero R, Eluf-Neto J, Ngelangel C, Franceschi S, et al. Evidence for Chlamydia trachomatis as a human papillomavirus cofactor in the etiology of invasive cervical cancer in Brazil and the Philippines. J Infect Dis. 2002;185(3):324-31.

69. Watts DH, Fazarri M, Minkoff H, Hillier SL, Sha B, Glesby M, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1–infected and high-risk HIV-1–uninfected women. J Infect Dis. 2005;191(7):1129-39.

70. Gillet E, Meys JF, Verstraelen H, Verhelst R, De Sutter P, Temmerman M, et al. Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and meta-analysis. PloS one. 2012;7(10):e45201.

71. Mendoza L, Mongelos P, Paez M, Castro A, Rodriguez-Riveros I, Gimenez G, et al. Human papillomavirus and other genital infections in indigenous women from Paraguay: a cross-sectional analytical study. BMC Infect Dis. 2013;13(1):531.

72. Parmar MT, Solanki HM, Gosaila VV. A study of prevalence of sexually transmitted infections & response to syndromic treatment among married women of reproductive age group in rural area of Parol primary health centre under Thane district. Glob J Med Public health. 2013;3(2):1-8.

73. Sankaranarayanan R, Thara S, Sharma A, Roy C, Shastri S, Mahe C, et al, Multicentre Study Group on Cervical Cancer Early Detection in India. Accuracy of conventional cytology: results from a multicentre screening study in India. J Med Screen. 2004;11(2):77-84.

74. Sasieni P, Adams J, Cuzick J. Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. Br J Cancer. 2003;89(1):88.

75. Jastania R, Geddie WR, Chapman W, Boerner S. Characteristics of apparently false-negative digene hybrid capture 2 high-risk HPV DNA testing. Am J Clin Pathol. 2006;125(2):223-8.

76. ICMR. National Centre for Disease Informatics and Research. National Cancer Registry Programme.
http://www.ncdirindia.org/All_Reports/PBCR_REPORT_2012_2014/index.htm
downloaded 12.6.2018

77. Sauvaget C, Nene BM, Jayant K, Kelkar R, Malvi SG, Shastri SS, et al. Prevalence and determinants of high-risk human papillomavirus infection in middle-aged Indian women. Sexually transmitted diseases. 2011;38(10):902-6.

78. Nene B, Jayant K, Arrossi S, Shastri S, Budukh A, Hingmire S, et al. Determinants of women's participation in cervical cancer screening trial, Maharashtra, India. Bull World Health Organ. 2007;85:264-72.

79. Sankaranarayanan R, Rajkumar R, Arrossi S, Theresa R, Esmy PO, Mahé C, et al. Determinants of participation of women in a cervical cancer visual screening trial in rural south India. Cancer Detect Prev. 2003;27(6):457-65.

80. Aswathy S, Quereshi MA, Kurian B, Leelamoni K. Cervical cancer screening: Current knowledge & practice among women in a rural population of Kerala, India. Indian J Med Res. 2012;136(2):205.

81. Yeole BB, Kumar AV, Kurkure A, Sunny L. Population-based survival from cancers of breast, cervix and ovary in women in Mumbai, India. Asian Pac J Cancer Prev. 2004;5(3):308-15.

82. Dutta S, Biswas N, Muhkherjee G. Evaluation of socio-demographic factors for non-compliance to treatment in locally advanced cases of cancer cervix in a rural medical college hospital in India. Indian J Palliat Care. 2013;19(3):158.

83. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. The Lancet Global Health. 2016;4(9):e609-16.

84. Bosch FX, De Sanjosé S. Human papillomavirus in cervical cancer. Curr Oncol Rep. 2002;4(2):175-84.

85. Bosch FX, Lorincz A, Muñoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002;55(4):244-65.

86. Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM, ALTS (Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study) Group, Plummer M. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis. 2007;195(11):1582-9.

87. HPV information centre. https://hpvcentre.net/hpvatglance.php downloaded on 12.8.2019.

88. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30:F55-70.

89. Fernandes JV, Araujo JD, Fernandes TA. Biology and natural history of human papillomavirus infection. Open Access J Clin Trials. 2013;5:1-2.

90. Einstein MH, Baron M, Levin MJ, Chatterjee A, Fox B, Scholar S, et al. Comparison of the immunogenicity of the human papillomavirus (HPV)-16/18 vaccine and the HPV-6/11/16/18 vaccine for oncogenic non-vaccine types HPV-31 and HPV-45 in healthy women aged 18–45 years. Hum Vaccin. 2011;7(12):1359-73.

91. Folschweiller N, Behre U, Dionne M, Durando P, Esposito S, Ferguson L, et al Long-term cross-reactivity against nonvaccine human papillomavirus types 31 and 45 after 2- or 3-dose schedules of the AS04-Adjuvanted human HPV-16/18 vaccine. J Infect Dis. 2019;219(11):1799-803.

92. Preisler S, Rebolj M, Ejegod DM, Lynge E, Rygaard C, Bonde J. Cross-reactivity profiles of hybrid capture II, cobas, and APTIMA human papillomavirus assays: split-sample study. BMC cancer. 2016;16(1):510.

93. Castle PE, Schiffman M, Burk RD, Wacholder S, Hildesheim A, Herrero R, et al. Restricted cross-reactivity of hybrid capture 2 with nononcogenic human papillomavirus types. Cancer Epidemiol Biomarkers. 2002;11(11):1394-9.

94. De Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. 2007;7(7):453-9.

95. Vinodhini K, Shanmughapriya S, Das BC, Natarajaseenivasan K. Prevalence and risk factors of HPV infection among women from various provinces of the world. Arch Gynecol Obstet. 2012;285(3):771-7.

96. Franceschi S, Rajkumar R, Snijders PJ, Arslan A, Mahe C, Plummer M, et al Papillomavirus infection in rural women in southern India. Br J Cancer. 2005;92(3):601.

97. Datta P, Bhatla N, Dar L, Patro AR, Gulati A, Kriplani A, et al. Prevalence of human papillomavirus infection among young women in North India. Cancer Epidemiol. 2010;34(2):157-61.

98. Gravitt PE, Paul P, Katki HA, Vendantham H, Ramakrishna G, Sudula M, et al, CATCH Study Team. Effectiveness of VIA, Pap, and HPV DNA testing in a cervical cancer screening program in a peri-urban community in Andhra Pradesh, India. PloS one. 2010;5(10):e13711.

99. Datta P, Bhatla N, Pandey RM, Dar L, Patro AR, Vasisht S, et al. Type-specific incidence and persistence of HPV infection among young women: a prospective study in North India. Asian Pac J Cancer Prev. 2012;13(3):1019-24.

100. Basu P, Roychowdhury S, Bafna UD, Chaudhury S, Kothari S, Sekhon R, et al. Human papillomavirus genotype distribution in cervical cancer in India: results from a multi-center study. Asian Pac J Cancer Prev. 2009;10(1):27-34.

101. Bhatla N, Lal N, Bao YP, Ng T, Qiao YL. A meta-analysis of human papillomavirus type-distribution in women from South Asia: implications for vaccination. Vaccine. 2008;26(23):2811-7.

102. Bhatla N, Dar L, Patro AR, Kumar P, Pati SK, Kriplani A, et al. Human papillomavirus-type distribution in women with and without cervical neoplasia in north India. Int J GynecolPathol: official journal of the International Society of Gynecological Pathologists. 2008;27(3):426.

103. Senapati R, Nayak B, Kar SK, Dwibedi B. HPV genotypes distribution in Indian women with and without cervical carcinoma: implication for HPV vaccination program in Odisha, eastern India. BMC Infect Dis. 2017;17(1):30.

104. Deodhar K, Gheit T, Vaccarella S, Romao CC, Tenet V, Nene BM, et al. Prevalence of human papillomavirus types in cervical lesions from women in rural Western India. J Med Virol. 2012;84(7):1054-60.

105. Castle PE, Schiffman M, Herrero R, Hildesheim A, Rodriguez AC, Bratti MC, et al. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J Infect Dis. 2005;191(11):1808-16.

106. Dutta S, Begum R, Mazumder D, Mandal SS, Mondal R, Biswas J, et al. Prevalence of human papillomavirus in women without cervical cancer: a population-based study in Eastern India. Int J Gynecol Pathol. 2012;31(2):178-83.

107. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clinical science. 2006;110(5):525-41.

108. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007;7(1):11.

109. Winer RL, Hughes JP, Feng Q, O'Reilly S, Kiviat NB, Holmes KK, et al. Condom use and the risk of genital human papillomavirus infection in young women. N Engl J Med. 2006;354(25):2645-54.

110. Brinton LA, Reeves WC, Brenes MM, Herrero R, DE Brllton RC, Gaitan E, et al. Parity as a risk factor for cervical cancer. Am J Epidemiol. 1989;130(3):486-96.

111. Mukherjee BN, Sengupta S, Chaudhuri S, Biswas LN, Maiti P. A case-control study of reproductive risk factors associated with cervical cancer. Int J Cancer. 1994;59(4):476-82.

112. Muñoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al, International Agency for Research on Cancer (IARC) Multicentric Cervical Cancer Study Group. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet. 2002;359(9312):1093-101.

113. Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas JO, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol Biomarkers. 2006;15(11):2148-53.

114. Moreno V, Bosch FX, Muñoz N, Meijer CJ, Shah KV, Walboomers JM, et al, International Agency for Research on Cancer (IARC) Multicentric Cervical Cancer Study Group. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. The Lancet. 2002;359(9312):1085-92.

115. Castle PE, Wacholder S, Lorincz AT, Scott DR, Sherman ME, Glass AG, et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. J Natl Cancer Inst. 2002;94(18):1406-14.

116. Marteau TM, Hankins M, Collins B. Perceptions of risk of cervical cancer and attitudes towards cervical screening: a comparison of smokers and non-smokers. Family practice. 2002;19(1):18-22.

117. Matsumoto K, Oki A, Furuta R, Maeda H, Yasugi T, Takatsuka N, et al. Tobacco smoking and regression of low-grade cervical abnormalities. Cancer sci. 2010;101(9):2065-73.

118. King CC, Jamieson DJ, Wiener J, Cu-Uvin S, Klein RS, Rompalo AM, et al. Bacterial vaginosis and the natural history of human papillomavirus. Infect Dis Obstet Gynecol. 2011;2011. doi:10.1155/2011/319460.

119. Silins I, Ryd W, Strand A, Wadell G, Törnberg S, Hansson BG, et al. Chlamydia trachomatis infection and persistence of human papillomavirus. Int J Cancer. 2005;116(1):110-5.

120. Mbulawa ZZ, Marais DJ, Johnson LF, Boulle A, Coetzee D, Williamson AL. Influence of human immunodeficiency virus and CD4 count on the prevalence of human papillomavirus in heterosexual couples. J Gen Virol. 2010;91(12):3023-31.

121. Mbulawa ZZ, Johnson LF, Marais DJ, Coetzee D, Williamson AL. The impact of human immunodeficiency virus on human papillomavirus transmission in heterosexually active couples. J Infect. 2013;67(1):51-8.

122. Singh DK, Anastos K, Hoover DR, Burk RD, Shi Q, Ngendahayo L, et al. Human papillomavirus infection and cervical cytology in hiv-infected and hiv-uninfected rwandan women. J Infect Dis. 2009;199(12):1851-61.

123. Prasad JH, Abraham S, Kurz KM, George V, Lalitha MK, John R, et al. Reproductive tract infections among young married women in Tamil Nadu, India. International family planning perspectives. 2005:73-82.

124. Garg S, Bhalla P, Sharma N, Sahay R, Puri A, Saha R, et al. Comparison of self-reported symptoms of gynaecological morbidity with clinical and laboratory diagnosis in a New Delhi slum. Asia Pac Popul J. 2001;16(2):75-92.

125. Bote MM, Shenoy AG. An epidemiological study to find out the prevalence of RTI/STI and various factors associated with it among ever married women of reproductive age group in an urban slum community of Mumbai. IOSR J Dent Med. 2014;13(3):9-15.

126. Prabha ML, Sasikala G, Bala S. Comparison of syndromic diagnosis of reproductive tract infections with laboratory diagnosis among rural married women in Medak district, Andhra Pradesh. Indian J Sex Transm Dis AIDS. 2012;33(2):112.

127. Balamurugan SS, Bendigeri ND. Community-based study of reproductive tract infections among women of the reproductive age group in the urban health training centre area in Hubli, Karnataka. Indian J Community Med. 2012;37(1):34.

128. Jindal N, Aggarwal A, Gill P, Sabharwal B, Sheevani BB. Community-based study of reproductive tract infections, including sexually transmitted infections, among the rural population of Punjab, India. Indian J Community Med. 2009;34(4):359.

129. Rao V, Savargaonkar D, Anvikar A, Bhondeley MK, Tiwary BK, Ukey M, et al. Reproductive Tract Infections in Tribal Women of Central India. Proceeding of National symphosium on Tribal health. 2006:275-7.

130. Chauhan V, Shah M, Thakkar S, Patel SV, Marfatia Y. Sexually transmitted infections in women: A correlation of clinical and laboratory diagnosis in cases of vaginal discharge syndrome. Indian dermatology online journal. 2014;5(Suppl 1):S1.

131. Vishwanath S, Talwar V, Prasad R, Coyaji K, Elias CJ, de Zoysa I. Syndromic management of vaginal discharge among women in a reproductive health clinic in India. Sexually Transmitted Infections. 2000;76(4):303-6.

132. Kaur H, Marwah P, Bajwa SK, Gill AK, Bal MS. Sexually transmitted infections (STIs) in gynae-outpatients: Experience from a tertiary health centre. Health. 2012;4(05):268.

133. Ray K, Bala M, Gupta SM, Khunger N, Puri P, Muralidhar S, et al. Changing trends in sexually transmitted infections at a Regional STD Centre in north India. Indian J Med Res. 2006;124:559-68.

134. Sharma S, Tiwari S, Paliwal V, Mathur DK, Bhargava P. Study of patterns of sexually transmitted diseases using a syndromic approach in the era of human immunodeficiency virus from a tertiary care hospital of the Northern India. Indian J Sex Transm Dis AIDS. 2015;36(2):158.

135. Caixeta RC, Ribeiro AA, Segatti KD, Saddi VA, Figueiredo Alves RR, dos Santos Carneiro MA, et al. Association between the human papillomavirus, bacterial vaginosis and cervicitis and the detection of abnormalities in cervical smears from teenage girls and young women. Diagnostic cytopathology. 2015;43(10):780-5.

136. Altuglu I, Terek MC, Ozacar T, Ozsaran AA, Bilgiç A. The prevalence of human papilloma virus DNA in women with mucopurulent endocervicitis. European journal of gynaecological oncology. 2002;23(2):166-8.

137. Stephen E. Hawes, Nancy B. Kiviat, Are Genital Infections and Inflammation Cofactors in the Pathogenesis of Invasive Cervical Cancer? JNCI. 2002;94(21):1592-93. doi.org/10.1093/jnci/94.21.1592

138. Ghosh I, Muwonge R, Mittal S, Banerjee D, Kundu P, Mandal R, et al. Association between high risk human papillomavirus infection and co-infection with Candida spp. and Trichomonas vaginalis in women with cervical premalignant and malignant lesions. J Clin Virol. 2017;87:43-8.

139. Lazenby GB, Taylor PT, Badman BS, Mchaki E, Korte JE, Soper DE, et al. An association between Trichomonas vaginalis and high-risk human papillomavirus in rural Tanzanian women undergoing cervical cancer screening. Clinical therapeutics. 2014;36(1):38-45.

140. Feng RM, Wang MZ, Smith JS, Dong L, Chen F, Pan QJ, et al. Risk of high-risk human papillomavirus infection and cervical precancerous lesions with past or current trichomonas infection: a pooled analysis of 25,054 women in rural China. J Clin Virol. 2018;99:84-90.

141. Rodriguez-Cerdeira C, Sanchez-Blanco E, Alba A. Evaluation of association between vaginal infections and high-risk human papillomavirus types in female sex workers in Spain. ISRN obstetrics and gynecology. 2012;2012.

142. Ghosh I, Ghosh P, Bharti AC, Mandal R, Biswas J, Basu P. Prevalence of human papillomavirus and co-existent sexually transmitted infections among female sex workers, men having sex with men and injectable drug abusers from eastern India. Asian Pac J Cancer Prev. 2012;13(3):799-802.

143. Qiagen. hc2 HIGH-RISK TEST. Qiagen Gaithersburg, Inc.US. 2008.

144. Malloy C, Sherris J, Herdman C. HPV/DNA testing: technical and programmatic issues for cervical cancer prevention in low-resource settings. Dec 2000.

145. Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, et al Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119(5):1095-101.

146. Huang YK, You SL, Yuan CC, Ke YM, Cao JM, Liao CY, et al. Long-term outcomes of high-risk human papillomavirus infection support a long interval of cervical cancer screening. Br J Cancer. 2008;98(5):863.

147. Elfström KM, Smelov V, Johansson AL, Eklund C, Nauclér P, Arnheim-Dahlström L, et al. Long term duration of protective effect for HPV negative women: follow-up of primary HPV screening randomised controlled trial. BMJ. 2014;348:g130.

148. Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH, et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine. 2008;26:K29-41.

149. Arbyn M, Sankaranarayanan R, Muwonge R, Keita N, Dolo A, Mbalawa CG, et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. Int J Cancer. 2008;123(1):153-60.

150. Larsen B, Monif GR. Understanding the bacterial flora of the female genital tract. Clinical Infectious Diseases. 2001;32(4):e69-77.

151. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol. 1991;29(2):297-301.

152. Money D. The laboratory diagnosis of bacterial vaginosis. Can J Infect Dis Med Microbiol. 2005;16(2):77-9.

153. Unemo M, Ballard R, Ison C, Lewis D, Ndowa F, Peeling R. Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus. VII World Health Organization.2013.

154. Centers for Disease Control and Prevention. Disease characterised by urethritis and cervicitis.http://www.cdc.gov/std/tg2015/urethritis-and-cervicitis.htm. Downloaded on 23.5.2019.

155. Brunham RC, Paavonen J, Stevens CE, Kiviat N, Kuo CC, Critchlow CW, et al. Mucopurulent cervicitis—the ignored counterpart in women of urethritis in men. N Engl J Med. 1984;311(1):1-6.

156. Patel DA, Burnett NM, Curtis KM. Reproductive tract infections. reproductive health epidemiology series-Module 3. US Department of Health and Human Services, Centre for Disease Control and Prevention, National Centre for Chronic Disease and Health Promotion, Division of Reproductive Health, Atlanta, Georgia, USA. 2003.

157. Tornesello ML, Buonaguro L, Giorgi-Rossi P, Buonaguro FM. Viral and cellular biomarkers in the diagnosis of cervical intraepithelial neoplasia and cancer. BioMed research international. 2013;2013. http://dx.doi.org/10.1155/2013/519619

158. Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol. 2005;32:43-51.

159. Sharma S, Gupta BP. The prevalence of reproductive tract infections and sexually transmitted diseases among married women in the reproductive age group in a rural area. Indian J Community Med: official publication of Indian Association of Preventive & Social Medicine. 2009;34(1):62.

160. Ratnaprabha GK, Thimmaiah S, Johnson AR, Ramesh N. Prevalence and awareness of reproductive tract infections among women in select underprivileged areas of Bangalore city. Int J Med Sci Public Health. 2015;4(12):1691-96.

161. Bhilwar M, Lal P, Sharma N, Bhalla P, Kumar A. Prevalence of reproductive tract infections and their determinants in married women residing in an urban slum of North-East Delhi, India. Journal of natural science, biology, and medicine. 2015;6(Suppl 1):S29.

162. Nasirian M, Baneshi MR, Kamali K, Haghdoost AA. Population-based survey on STI-associated symptoms and health-seeking behaviours among Iranian adults. Sex Transm Infect. 2016;92(3):232-9.

163. Das S, Dasgupta A. Community based study of reproductive tract infections among women of the reproductive age group in a rural community of Eastern India. Int J Community Med Public health. 2018;6(1):330-6.

164. Chaudhary N, Kalyan R, Agarwal J, Singh M, Qureshi S. Evaluation of risk factors in women attending a sexually transmitted infection clinic at a tertiary care centre. International Journal of Research in Medical Sciences. 2018;6(7):2332.

165. Rizwan SA, Rath RS, Vivek G. KAP study on sexually transmitted infections/Reproductive tract infections (STIs/RTIs) among married women in rural Haryana. Indian dermatology online journal. 2015;6(1):9.

166. Centre for Disease Control and Prevention. Bacterial Vaginosis, https://www.cdc.gov/std/tg2015/bv. Downloaded on 19th June 2018.

167. Peipert JF, Montagno AB, Cooper AS, Sung CJ. Bacterial vaginosis as a risk factor for upper genital tract infection. Am J Obstet Gynecol. 1997;177(5):1184-7.

168. Stern J, Givan A, Gonzalez J, Harper D, White H, Wira C. Leukocytes in the cervix: a quantitative evaluation of cervicitis. Obstetrics & Gynecology. 1998;91(6):987-92.

169. Nayar R, Wilbur DC. The pap test and Bethesda 2014. Acta cytologica. 2015;59(2):121-32.

170. Prey M. Routine Pap smears for the diagnosis of bacterial vaginosis. Diagnostic cytopathology. 1999;21(1):10-3.

171. Eriksson K, Forsum U, Bjørnerem A, PLATZ-CHRISTENSEN JJ, Larsson PG. Validation of the use of Pap-stained vaginal smears for diagnosis of bacterial vaginosis. Apmis. 2007;115(7):809-13.

172. Davis JD, Connor EE, Clark P, Wilkinson EJ, Duff P. Correlation between cervical cytologic results and Gram stain as diagnostic tests for bacterial vaginosis. Am J Obstet Gynecol. 1997;177(3):532-5.

173. Tokyol Ç, Aktepe OC, Cevrioğlu AS, Altındiş M, Dilek FH. Bacterial vaginosis: comparison of Pap smear and microbiological test results. Modern pathology. 2004;17(7):857.

174. Karani A, De Vuyst H, Luchters S, Othigo J, Mandaliya K, Chersich MF, et al.The Pap smear for detection of bacterial vaginosis. Int J Gynaecol Obstset. 2007;98:20-23.

175. Greene III JF, Kuehl TJ, Allen SR. The papanicolaou smear: inadequate screening test for bacterial vaginosis during pregnancy. Am J Obstet Gynecol. 2000;182(5):1048-9.

176. Centers for Disease Control and Prevention.https://www.cdc.gov/std/tg2015/chlamydia.htm downloaded on 13.6.2019

177. Centres for Disease Control and Prevention. https://www.cdc.gov/std/tg2015/gonorrhea.htm downloaded on 13.6.2019

178. Bohbot JM, Vicaut E, Fagnen D, Brauman M. Treatment of bacterial vaginosis: a multicenter, double-blind, double-dummy, randomised phase III study comparing secnidazole and metronidazole. Infect Dis Obstet Gynecol. 2010;2010.doi:10.1155/2010/705692

179. Bote MM, Bedre RC, Solanki HB, Shenoy AG, Suryawanshi SR. Syndromic diagnosis vs laboratory diagnosis of reproductive tract infection among married women of reproductive age group in Urban slum of Mumbai. Ntl J of Community Medicine. 2015;6:513-8.

180. Ray K, Muralidhar S, Bala M, Kumari M, Salhan S, Gupta SM, et al. Comparative study of syndromic and etiological diagnosis of reproductive tract infections/sexually transmitted infections in women in Delhi. Int J infect Dis. 2009;13(6):e352-9.

181. Tankhiwale SS, Chavan SP. Comparative study of syndromic and etiological diagnosis of sexually transmitted infection except human immunodeficiency virus in sexually transmitted infection and reproductive tract infection clinic attendees in central India. Int J Med Public Health. 2013;3(4).347-350.

182. Barry MS, Ba Diallo A, Diadhiou M, Mall I, Gassama O, Ndiaye Guèye MD, et al. Accuracy of syndromic management in targeting vaginal and cervical infections among symptomatic women of reproductive age attending primary care clinics in Dakar, Senegal. Tropical Medicine & International Health. 2018;23(5):541-8.

183. Pickering JM, Whitworth JA, Hughes P, Kasse M, Morgan D, Mayanja B, et al. Aetiology of sexually transmitted infections and response to syndromic treatment in southwest Uganda. Sexually transmitted infections. 2005;81(6):488-93.

184. Kore S, Pandole A, Kulkarni S, Puthuraya S, Kamat S, Ambiye+ VR. Original/research: Syndromic management of vaginal discharge our experience. Sited on. 2013;9.

185. Rodríguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle PE, et al, Proyecto Epidemiológico Guanacaste Group. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008;100(7):513-7.

186. Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii Human Papillomavirus Cohort Study. Cancer research. 2008;68(21):8813-24.

187. Dalstein V, Riethmuller D, Prétet JL, Le Bail Carval K, Sautière JL, Carbillet JP, et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int J Cancer. 2003;106(3):396-403.

188. Molano M, van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am J Epidemiol. 2003;158(5):486-94.

189. Rosa MI, Fachel JM, Rosa DD, Medeiros LR, Igansi CN, Bozzetti MC. Persistence and clearance of human papillomavirus infection: a prospective cohort study. Am J Obstet Gynecol. 2008;199(6):617-e1.

190. Wheeler CM, Greer CE, Becker TM, Hunt WC, Anderson SM, Manos MM. Short-term fluctuations in the detection of cervical human papillomavirus DNA. Obstetrics & Gynecology. 1996;88(2):261-8.

191. Basu P, Dutta S, Begum R, Mittal S, Dutta PD, Bharti AC, et al. Clearance of cervical human papillomavirus infection by topical application of curcumin and curcumin containing polyherbal cream: a phase II randomized controlled study. Asian Pac J Cancer Prev. 2013;14(10):5753-9.

192. Moscicki AB, Schiffman M, Kjaer S, Villa LL. Updating the natural history of HPV and anogenital cancer. Vaccine. 2006;24:S42-51.

193. Mariani L, Venuti A. HPV vaccine: an overview of immune response, clinical protection, and new approaches for the future. Journal of translational medicine. 2010;8(1):105.

194. Bulkmans NW, Berkhof J, Bulk S, Bleeker MC, Van Kemenade FJ, Rozendaal L, et al. High-risk HPV type-specific clearance rates in cervical screening. Br J Cancer. 2007;96(9):1419.

195. Kim JW, Song SH, Jin CH, Lee JK, Lee NW, Lee KW. Factors affecting the clearance of high-risk human papillomavirus infection and the progression of cervical intraepithelial neoplasia. J Int Med Res. 2012;40(2):486-96.

196. Gillio-Tos A, De Marco L, Carozzi FM, Del Mistro A, Girlando S, Burroni E, et al. Clinical impact of the analytical specificity of the hybrid capture 2 test: data from the New Technologies for Cervical Cancer (NTCC) study.J Clin Microbiol.. 2013;51(9):2901-7.

197. Sargent A, Bailey A, Almonte M, Turner A, Thomson C, Peto J, et al. Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. Br J Cancer. 2008;98(10):1704.

198. Van Ham MA, Melchers WJ, Hanselaar AG, Bekkers RL, Boonstra H, Massuger LF. Fluctuations in prevalence of cervical human papillomavirus in women frequently sampled during a single menstrual cycle. Br J Cancer. 2002;87(4):373.

199. Viscidi RP, Schiffman M, Hildesheim A, Herrero R, Castle PE, Bratti MC, et al. Seroreactivity to human papillomavirus (HPV) types 16, 18, or 31 and risk of subsequent

HPV infection: results from a population-based study in Costa Rica. Cancer Epidemiol Biomarkers. 2004;13(2):324-7.

200. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine. 2006;24:S4-15.

201. Trottier H, Ferreira S, Thomann P, Costa MC, Sobrinho JS, Prado JC, et al. Human papillomavirus infection and reinfection in adult women: the role of sexual activity and natural immunity. Cancer research. 2010;70(21):8569-77.

202. Kjaer SK, Svare EI, Worm AM, Walboomers JM, Meijer CJ, Van den Brule AJ. Human papillomavirus infection in Danish female sex workers: decreasing prevalence with age despite continuously high sexual activity. Sexually transmitted diseases. 2000;27(8):438-45.

203. Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia?: A meta-analysis. Sex Transm Dis. 2002;29(11):725-35.

204. Christopher A. Hearing addresses condoms for HPV prevention. J Natl Cancer Inst. 2004;96(13):985. https://doi.org/10.1093/jnci/96.13.985.

205. Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16(1):1-7.

ANNEXURE 1

ANNEXURE 1.

Table 1. Definitions for therapeutic response on per speculum examination.

Category	**Clinical findings**
Cured	No clinical signs of cervicitis.
Better	Clinically signs of cervicitis decreased as compared to previous clinically finding by more than 75%.
Failure	Clinically no improvement in signs of cervicitis as compared to baseline findings.

Adapted and modified. Taylor et al. Prevalence and treatment outcome of cervicitis of unknown etiology.

Table 2. Working table for grading of pus cells on Gram stain per high power field used to report Non Gonococcal infection (NGI).

Category	Pus cell on High power field on Gram stain slide*
Grade 1	Less than 10 pus cell/HPF
Grade 2	11-25 pus cell/HPF
Grade 3	26-40 pus cell/HPF
Grade 4	Uncountable pus cells/HPF.

* The above working table was designed for the microbiologist to report the pus cell counts as they were blinded to clinical findings.

Table 3. Studies reporting fluctuations and clearance of oncogenic HPV at various time interval.

Sr.No.	Author year	Place Cohort	Sample size	Baseline HR HPV positive (HPV Positive)	Age Median age (Range in yrs)	Test used	Clearance reported	Probable causes reported by author for HPV fluctuations & clearance
1)	Achim Schneider Et al 1992	Panel study Chicago	21	4	23.6 yrs (19 - 32)	PCR type 16 Probe	50% at 5 weeks	Fluctuation 1.Due to hormonal phase of cycle. 2.Viral load fluctuation.
2)	MAPC Ham et al 2002	Fertility clinic Netherland	20	3	33.8 yrs (22 – 43)	PCR	33.3% at 5 days	Fluctuation 1.Cell adequacy 2.Variation in viral load 3.Harmones effect
3)	Moscicke AB et al 1993	Young woman cohort California	500	17(27)	17.75yrs (13 – 19)	Viral Pap	82% at 6 months	Fluctuation 1.can occur intermittently due to immunity control causing a latent infection. 2.Viral load fluctuations.
4)	Wheeler CM et al 1996	Mexico	72	18	27 yrs (18 – 35)	PCR	33.3% at 7 days	Fluctuation 1.Host difference. 2.Sampling error. 3.Assay detection limits. 4.Level and pattern of HPV replication. 5.New infection.
5)	Anna Cecilia et al 2008	Guanacaste Costa Rica cohort	599	60	30 yrs & above	PCR	33% at 3 months	1.Clearance rates are affected by screening intervals.
6)	Moscicke AB 1992	Young woman cohort California	500	44 (76)	17.75 yrs (13-21)	Viral Pap	43.2% at 4.6 months	Clearance 1.low viral load 2.Type specific HPV. 3.Host immunity
7)	Maria Rosa et al. 2008	South Brazil cohort	501	177	43 (SD+ 13)	PCR (16,18,31)	80.8% at 19 months	Clearance 1.Treating STIs. 2.Regular screening by Pap.
8)	Marc Goodman et al. 2008	Hawai cohort	972	243	33 (18 – 85)	PCR	55% at 7 months	Clearance 1.No consensus on clearance definition. 2.Increase clearance with older age (change findings) and less than Para3
9)	Veronique Dalstein et al 2003	French cohort	781	257	35. 7 yrs (16 – 76)	HC2	50.6% at 7.5 months	Clearance 1.Viral load 2. Cytology findings
10)	Present study	Mumbai	508	Case-14.4% Control-5.3% (at baseline)	Case-37 yrs Control-38 yrs (30-50 yrs)	HC2	Case - 25.7% at 13 days Control-46.2% at 11 days	Clearnce RLU factor Presence/absence of RTIs

Note: We looked into fluctuation and clearance of High risk HPV at different interval of time. HPV is a transient infection and a short-term HPV detection is highly variable within an individual. Fluctuations can be due to resolution of HPV due to immunity or acquisition of new HPV, viral load fluctuation. Clearance rates are affected by screening intervals, type specific HPV, Viral loads, cytology findings, treating STIs and the host immunity.

ANNEXURE 2

Annexure- 2. Academics and publications.

Courses & credit Seminars 2013-2019

A. Courses attended

1.Fundamental of Biostatistics, Principles of Epidemiology & SPSS (Nov 2013)
2.Molecular Epidemiology Course (April 2014)
3.Multiple Linear Regression, Logistic Regression & Survival Analysis (Nov 2014)
4.Good Clinical Practice- Advance Short Course (Feb 2016, Nov 2018)

B. workshop/Conference attended as delegate

1.XVth Annual meeting on 'Evidence Based Management of Cancers in India (Feb2017)
2.Colposcopy & Cervical Cytopathology Workshop (Oct 2017)

C. Workshop/Conference attended as Faculty

1.CME on 'Women's Cancer Prevention, Screening and Early Detection' (Feb 2016)
2.CME by Indian Association of Physiotherapists on 'Prevention and Screening in Cancer of Cervix and Breast'(March 2016)
3.17th Joint State Conference of IAPSM & IPHA, MHIAPSMIPHACON-2016 in association with Mumbai branch of IAPSM-2016.(Feb 2016)
3.Scientific paper presented at Kolkata Health Summit. (Sept 2017)
4.Certificate course 'Overview in Preventive Oncology' (2014-2018) - 22
5.Workshop on 'Tobacco Control and Cessation' (2014-2018) - 5

D.Poster Presentation

1.Scientific Symposium 'Frontiers in Epidemiology'(March 2017)

E. Paper publication

Part of thesis

1. 'Reliability of conventional Pap smear in diagnosing Bacterial Vaginosis among women with clinical genital infection.' (accepted South Asian Journal of Cancer)

Other publication

1. 'Comparative Evaluation of Results of Pap smears and HPV Hybrid Capture 2 Performed on cervical sample before and after application of acetic acid. (published- International Journal of Health Sciences and research)

E.Ongoing training

Colposcopy training by 'The International Federation of Cervical Pathology and Colposcopy.

IJHSR International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Comparative Evaluation of Results of Pap Smears and HPV Hybrid Capture 2 Performed On Cervical Samples Before and After Application of Acetic Acid

Dr Anand Kavita V[1], Dr Mishra Gauravi A[1], Dr Pimple Sharmila A[1], Dr Deodhar Kedar K[2], Dr Kelkar Rohini S[3], Ms Dighe Swati[4], Dr. Vasundhara Y Kulkarni[1]

*PhD Scholar, [1]Dept of Preventive Oncology, [2]Dept. of Pathology, [3]Dept of Microbiology, [4]Dept of Cytopathology,
Tata Memorial Hospital, Parel, Mumbai.

Corresponding Author: Dr Mishra Gauravi A

ABSTRACT

Aim: To assess the feasibility of triaging VIA (Visual inspection with Acetic Acid) positive women with a test with higher specificity, either Pap or HPV DNA test (Human Papilloma Virus) in the same sitting at community level. This strategy if successful shall prevent unnecessary referrals to tertiary care in resource constrained settings and also increase the programmatic yield.

Objective: To investigate, whether the 5% acetic acid, used in VIA screening will compromise the cellularity of Pap test or results of HPV DNA test.

Materials/ Methods: Fifty women were randomised to either HPV test arm or Cytology test arm. The 25 women in HPV DNA arm underwent a HPV DNA HC2 test before and five minutes after application of 5% acetic acid. In cytology arm 25 women underwent Pap test before and five minutes after application of 5% acetic acid.

Conclusions: According to results of this study, 5% acetic acid affected the cellularity of cytology smears and may affect interpretation of dysplastic smears. HPV DNA test results remained unaffected by acetic acid. These results suggest that HPV HC2 may perhaps be a better triage test for VIA positive women. But this needs confirmation with a larger sample size. These findings are extremely crucial in the scenario that National Cancer Control Programme in many low-income countries including India are adopting VIA based screening and there is unavailability of resources to handle the large number of false positives emerging from positive VIA test.

Key words: Acetic acid, Pap smear, HPV DNA test.

INTRODUCTION

Though cervical cancer has a preventable precancerous stage that can be diagnosed by simple and effective screening methods, cervical cancer still remains third common cancer in women globally and is the second most common cancer among Indian women. [1]

In order to reduce the cervical cancer burden, it is important to screen the women and detect the cervical cancers in pre-cancerous stage when complete cure is still possible. [2]

Pap smear has decreased the cervical cancer mortality among developed countries, [3,4] but in Low Middle Income Countries (LMIC) similar results are not evident for various reasons. It is difficult to implement cytology-based screening programme in LMIC mainly due to issues of lack of awareness among women, uneven distribution of health care facilities, need of infrastructure in terms of laboratory, a

Reliability of conventional Papanicolaou smear in diagnosing bacterial vaginosis among women with clinical genital infection

Kavita Vivek Anand, Sharmila Anil Pimple, Gauravi A. Mishra, Rupali V. Sahare[1], Saleem Pathuthara[2], Kedar K. Deodhar[3], Surendra S. Shastri[4]

Abstract

Objective: Bacterial vaginosis (BV) is a common reproductive tract infection (RTI) reported among Indian women. BV can influence the persistence of high-risk oncogenic human papillomavirus, a causative factor for cervical cancer. BV and cervical cancer are major public health issues in a developing country like India. It becomes important for a resource-constrained country like India with poor healthcare access to implement control measures to screen and treat RTI in an attempt to prevent the risk for cervical cancer. Papanicolaou (Pap) smear is an established screening tool for cervical cancer and the diagnosis of RTIs, forms a part of its evaluation. The present study explores the validity of conventional Pap smear in diagnosing BV. **Methodology:** Pap smear and Gram stain smear were collected for 254 women with clinically evident cervicitis/cervicovaginitis (genital infection). Using the Nugent score on Gram stain as a gold standard, we determined the sensitivity and specificity of Pap smear to diagnose BV. **Results:** The overall prevalence of BV in the study population was 44% using the Nugent score. Pap smear showed sensitivity and specificity of 70.9%. (CI- 61.5% - 79.2%) and 56.8% (CI – 48.2%–65.2%), respectively. The positive predictive value of Pap smear to diagnose BV was 56.5% (CI – 47.8%–64.9%), and the negative predictive value was 71.2% (CI – 61.8%–79.4%). **Conclusion:** In the present study, conventional Pap smear demonstrates good accuracy to detect BV. Pap testing for cervical cancer screening can additionally serve as an effective screening tool for diagnosing BV among women with genital infection in healthcare settings.

Key words: Bacterial vaginosis, cervicitis, human papillomavirus, Nugent score, Papanicolaou smear

Introduction

The midterm report of the National AIDS Control Organization (NACO), India, which was based on a review of published and unpublished population-based studies, reported bacterial vaginosis (BV) to be the most common reproductive tract infections (RTIs) among Indian women.[1] A huge burden of symptomatic/asymptomatic BV is reported worldwide.[2,3] There is emerging evidence of a strong association between cervicitis and BV.[4,5]

BV infection needs to be addressed as it has the potential to cause maternal morbidity due to its association with common conditions such as pelvic inflammatory disease, chorioamnionitis, and preterm labor in women.[6] Due to the change in vaginal microbiological flora and inflammation associated with BV, it may help in acquiring and transmitting human papillomavirus (HPV) infection which is the main cause for cervical cancer. The inflammation of the cervix causes break in the cervical epithelium helping the HPV to gain entry in the actively proliferating basal cells of the cervical epithelium.[7] Inflammation is known to cause DNA damage of the host cell leading to the integration of viral DNA, which leads to gradual progression of HPV infection to microinvasion and invasive cervical cancer. BV may serve as a cofactor for the persistence of high-risk HPV, thus diagnosing and treating BV infection may help to reduce the risk of cervical cancer in women.[8-12]

Papanicolaou (Pap) smear beside an established screening test for the detection of cervical precancerous lesions is also posed to diagnose sexually transmitted infections (STI)/reproductive transmitted infections (RTIs).[13] Pap smear has the potential to serve as a cost-effective tool for dual screening of BV and cervical cancer which are the most common cause of morbidity and mortality reported among Indian women.[1,14] Among the RTIs, the role of Pap smear to diagnose BV has conflicting results reported from several developed and developing countries.[3,15-20] There is limited evidence from India about the accuracy of Pap smear in detecting BV. To the best of our knowledge, the only community-based study conducted from India demonstrated encouraging results of Pap smear to diagnose BV infection with sensitivity of 78%.[3] The present study evaluates the accuracy of Pap smear to diagnose BV infection in women with clinically evident genital infection using the Nugent score on Gram-stained smear as the gold standard.[21-23] The microbiology evaluation to diagnose BV infection by the Nugent score done for the women enrolled in the present study is a part of the main study which looks into "Performance of HPV DNA test in the presence of coinfection with common RTIs".

Methodology

The study design is a prospective blinded cross-sectional study of women in the reproductive age group who presented for routine cervical cancer screening in a tertiary care institute between August 2016 and August 2018.

The inclusion criteria for the study were nonpregnant women in the age group of 30–50 years and having clinically evident cervicitis/cervicovaginitis (genital infection) on per speculum examination. The case definition of cervicitis was unhealthy cervix with the presence of cervical erythema and inflammation that bleeds on touch with mucopurulent/purulent discharge. Among 3900 women visiting the department of cancer screening during the period from August 2016 to

Department of Preventive Oncology, Tata Memorial Centre, Affiliated to Homi Bhabha National Institute, [1]Department of Microbiology, R. N. Cooper General Hospital, Departments of [2]Cytopathology and [3]Pathology, Tata Memorial Hospital, Mumbai, Maharashtra, India, [4]Department of Health Disparities Research, Division of Cancer Prevention and Population Science, MD Anderson Cancer Centre, Houston, USA

Correspondence to: Dr. Sharmila Anil Pimple, E-mail: drsharmilapatil@yahoo.com, pimplesa@tmc.gov.in

Access this article online

Quick Response Code:

Website: www.sajc.org

DOI: 10.4103/sajc.sajc_421_18

How to cite this article: Anand KV, Pimple SA, Mishra GA, Sahare RV, Pathuthara S, Deodhar KK, *et al*. Reliability of conventional Papanicolaou smear in diagnosing bacterial vaginosis among women with clinical genital infection. South Asian J Cancer 2020;9:13-6.

Gmail

Kavita Anand <drkavitaanand@gmail.com>

Manuscript #(Cytojournal_74_2019) Accepted for Publication

CytoJournal <no-reply@editorialassist.com> Fri, Dec 27, 2019 at 7:12 AM
Reply-To: CytoJournal <editor@cytojournal.com>
To: Kavita Anand <drkavitaanand@gmail.com>

CytoJournal

ScientificScholar®
Knowledge is power
Publisher of Scientific Journals
"Share, Learn and Improve"

Dear Dr. Anand ,

We are glad to inform that your manuscript #Cytojournal_74_2019 titled "Detection of Rare Parasite on Pap Smear" is now accepted for publication in CytoJournal.

Before publication, our production team will check the format of your manuscript to ensure that it conforms to the standards of the journal. They may be in touch with you shortly to request any necessary changes, or to confirm that none are required.

Best wishes,
Dr. Vinod Shidham
Editor-in-Chief
CytoJournal

ANNEXURE 3

BRIHANMUMBAI MANAGARPALIKA

H.B.T. MEDICAL COLLEGE & DR.R.N.COOPER MUNICIPAL GEN.HOSPITAL, JUHU, MUMBAI-56

INSTITUTIONAL ETHICS COMMITTEE

Ho/10729/RNCH Tel no: 26207258 ; fax no: 26205897

18/7/2016.

To,

DR.KAVITA ANAND, DR REENA WANI

The Ethics Committee of HBT Medical College & Dr.R. N. Cooper Hospital in its meeting has reviewed and discussed your research study titled **"PERFORMANCE OF HPVDNA TEST IN PRESENCE OF CO-INFECTION WITH COMMON RTIS"**

The following members of committee were present at meeting on 15 /07/2016

Chairman	DR A.J.THAKUR	(Orthopedic consultant)
Secretary	DR.Vinod Gite	(Assist. Prof. ENT)
Members	Dr. Naina Dalvi	PROF. ANAESTHESIA
	Dr. Vinay Patke	PROF. BIOCHEMISTRY
	Dr. Satish Kale	Honorary Orthopedic Consultant
	Advocate Sanjeev Agawane	Legal Adviser
	Mrs.Vidula Patil	Medical social worker
	Mukesh Garg	LAY PERSON
	DR M. M. KAMAT	HON. DEPT OF SURGERY

The Ethics Committee approves the protocol in its presented form. You are requested to submit a final report of your project at the end of study.

DATE:

DR. R.N. COOPER HOSPITAL JUHU, MUMBAI-56. ETHICS COMMITTEE

Yours sincerely,

Chairman

Institutional Ethics Committee

TATA MEMORIAL CENTRE
टाटा स्मारक केन्द्र
TATA MEMORIAL HOSPITAL
टाटा स्मारक अस्पताल

Institutional Ethics Committee
w.e.f. 15th October, 2013

INSTITUTIONAL REVIEW BOARD

IEC/0518/1671/001

May 7, 2018

To,
Dr. Sharmila Pimple,
Principal Investigator,
Department of Preventive Oncology
Tata Memorial Hospital

Ref: Project No. 1671:- "Performance of HPV DNA Test in presence of co-infection with common RTIs"

Dear Dr. Pimple,

The continuing review application following documents for the above referenced project was discussed during the Institutional Ethics Committee-II (IEC-II) meeting held on **04/05/2018 at 8.30 am in Institutional Review Board Meeting Room, Main Bldg, 3rd Floor, Tata Memorial Hospital.**

The following members of the Institutional Ethics Committee-II were present:

Sr. No.	Name	Position	Affiliation	Gender	Expertise
1	Dr.Sudeep Shah	Chairperson	Consultant in Gastroenterology surgery, Hinduja Hospital	Male	Surgeon
2	Dr.Arun Bhatt	Co- Chairperson	Consultant – Clinical Research and Development	Male	Clinician
3	Dr.Girish Chinnaswamy	Member Secretary & Clinician	Professor, Dept. of Medical Oncology, TMH	Male	Medical Oncologist (Paediatrician)
4	Dr.Yashashri Shetty	Basic Medical Scientist	Associate Professor, Department of Pharmacology & Therapeutics, Seth GS Medical College & KEM Hospital	Female	Clinical Pharmacologist
5	Mr. KV Ganpathy	Layperson	CEO, Jeet Association for Support to Cancer Patients (JASCAP)	Male	Human Resource Management
6	Dr.Bindulakshmi P	Social scientist	Associate Professor, Advanced Centre for Women's Studies, School of Development Studies, Tata Institute of Social Sciences	Female	Gender Health and Rights and Mental Health and Disabilities
7	Ms. Tanisha Doshi	Legal expert	Associate, Hariani & Co, Mumbai	Female	Legal expert
8	Dr. Neelam Shirsat	Scientific Member	Scientific Officer, ACTREC	Female	Basic Scientist
9	Dr. Ashwini Budrukkar	Clinician	Professor, Dept. of Radiation	Female	Radiation

IRB Office
Dr. E. Borges Marg, Parel,
Mumbai - 400 012. India
Phone : 022-2417 7262
Fax : 022-22 2415 4005

E-mail : tmhethics@gmail.com
Website : http://tmc.gov.in

आय.आर.बी. ऑफीस
डॉ. ई. बोर्जेस मार्ग, परेल
मुंबई - ४०० ०१२. भारत
दूरभाष : ०२२-२४१७ ७२६२
फैक्स : ०२२-२४१५ ४००५

Sr. No.	Name	Position	Affiliation	Gender	Expertise
			Oncology, TMH		Oncologist
10	Dr.Priya Ranganathan	Member & Jt. Secretary Data Safety Monitoring Unit (DSMU) & Clinician	Professor, Dept. of Anaesthesia, TMH	Female	Anesthetist
11	Dr.Nita Nair	Clinician	Associate Professor, Dept. of Surgery, TMH	Female	Surgeon
12	Dr. Suyash Kulkarni	Clinician	Professor & Head, Division of Interventional Radiology, TMH	Male	Interventional Radiologist
13	Dr. Vikas Ostwal	Clinician	Associate Professor, Dept. of Medical Oncology, TMH	Male	Medical Oncologist
14	Dr.Mukta Ramadwar	Clinician	Professor, Dept. of Pathology, TMH	Female	Basic Medical Scientist (Pathologist)
15	Dr.Nilendu Purandare	Clinician	Professor, Dept. of Nuclear Medicine	Male	Radiologist

Status: IEC approved the continuation of the study till 13/06/2019. The Principal Investigator should submit continuing review application/annual status report on or before 13/04/2019. In order to ensure that there is no lapse in the IEC approval period, it is mandatory to submit study status report prior to lapse of study validity.

This decision was taken unanimously.

Neither PI nor any of proposed study team members participated during the decision making of the IEC.

Thanking you,

Yours faithfully,

Dr. Girish Chinnaswamy,
Member Secretary,
Institutional Ethics Committee-II

Annexure- 4. Baseline RLU/CO values among women with discrepancies findings.

Table 1. HC2 test results with RLU titers among women with HC2 positive test results on baseline visit and negative test results on a follow up visit.

Category	HC2 day 1	RLU titers	HC2 results day 14	RLU titers
Control	Positive	1.49	Negative	N/A
Cases	Positive	11.00	Negative	N/A
Control	Positive	8.00	Negative	N/A
Cases	Positive	27.00	Negative	N/A
Cases	Positive	1.45	Negative	N/A
Control	Positive	2.00	Negative	N/A
Cases	Positive	1.20	Negative	N/A
Cases	Positive	4.00	Negative	N/A
Control	Positive	4.00	Negative	N/A
Cases	Positive	1.37	Negative	N/A
Cases	Positive	2.00	Negative	N/A
Cases	Positive	1.20	Negative	N/A
Cases	Positive	4.00	Negative	N/A
Control	Positive	46.00	Negative	N/A
Control	Positive	4.00	Negative	N/A

Table 2- HC2 test results with RLU titers among women with HC2 negative test results on baseline visit and positive test results on a follow up visit.

Category	HC2 day 1	RLU titers	HC2 day 14	RLU titers
Cases	Negative	N/A	Positive	3.00
Cases	Negative	N/A	Positive	4.00
Cases	Negative	N/A	Positive	5.00
Control	Negative	N/A	Positive	3.00
Cases	Negative	N/A	Positive	9.00
Control	Negative	N/A	Positive	13.00
Cases	Negative	N/A	Positive	1624.00
Cases	Negative	N/A	Positive	2.00
Cases	Negative	N/A	Positive	153.00
Control	Negative	N/A	Positive	2879.00
Cases	Negative	N/A	Positive	97.00
Cases	Negative	N/A	Positive	1.38
Cases	Negative	N/A	Positive	2138.00
Control	Negative	N/A	Positive	4.00
Cases	Negative	N/A	Positive	2.00