RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Comparing Hpv Dna Pcr Test With Hpv Hc 2 For Screening Of Cancer Cervix

KEYWORDS: HPV DNA, PAP, hc2 technology, CIN.

Dr Jyotsna Sanjay Potdar	Dr Pushplata Naphade
MD (OBG) Asso Professor, SKNMC & GH, Narhe, Pune(MS)	MD(OBG). Professor & Head, Department of Obstetrics & Gynecology, SKNMC & GH, Narhe, Pune (MS).

ABSTRACT *OBJECTIVE- To compare sensitivity of HPV DNA PCR with HPV HC 2 in screening for cancer cervix.*

METHODS-

Part1- *Study period for HPV DNA PCR from June 2009 to Dec 2010 at Shrimati Kashibai Navale Medical College & General Hospital in the department of Obstetrics & Gynaecology. Five hundred women underwent PAP smear exam. Twenty seven women had abnormal PAP report. Women who had abnormal PAP report had HPV DNA PCR testing. Twelve women had HSIL & Fifteen women had LSIL reports. None of these 27 women had a positive HPV DNA PCR report.(1)*

Part2- *Study period from Jan 2012 to July 2012 at Shrimati Kashibai Navale Medical college & General Hospital where HPV DNA testing was done with HC-2 (hybrid capture -2).Five hundred women underwent PAP smear. Surprisingly (as with HPV PCR test) twenty seven women had abnormal PAP report. Nineteen women had LSIL report & eight women had HSIL report. Four women of LSIL group were HPV positive (20%) where as two out of eight women with HSIL report were HPV positive (20%).(2)This difference between results of two studies is statistically significant with p value of less than .o1.*

CONCLUSION- *Kit used for HPV DNA testing should be analysed before use for which detailed discussion is given for interpretation. The test kit we were using for HPV DNA PCR test had L1 as probe for test.L1 is lost during viral transformation.*

INTRODUCTION

As FOGSI initiative, after Ethical committee sanction a study was designed so as to test high risk women with abnormal PAP smear with HPV DNA PCR test. This initiative was taken to ensure that all women who walk in Gynaecological outpatient department undergo examination of cervix by PAP smear, visual inspection of cervix after application of acetic acid or Lugols Iodine for screening of CIN or examination of cervix with naked eye to exclude visible cancerous abnormalities of cervix. Tests were done to screen for cancer cervix as we do not have any Government of India initiative of screening of sexually active women for cancer cervix. Results are already obvious specially in urban areas where cancer registry is showing drop in number of cancer cervix. Our college is situated in Narhe village very near Pune city & drains many rural area patients as it is free hospital though a private medical college. Corporation & Government college hospitals are very far for them to access. Thus screening for cancer cervix was done very sincerely by us by any means as mentioned above and definitely by sensitizing future MBBS & MS doctors.

As HPV DNA PCR did not show any positive test among any of the LSIL or HSIL PAP reports it became obvious that though sensitivity reported is as high as 85% - 95% it did show same result in our study. Then we tested our diagnosed cancer cervix patient for HPV DNA PCR test and we found that they showed consistently positive result. Something was amiss that some part of viral part is lost during premalignant stage which is regained during cancerous transformation.(3) The analytical sensitivity of PCR methods can be less than 10 copies of HPV DNA so this result was totally unexpected.

Justification of study- Finding answers to post launch dilemmas when clinicians use kits that do not give expected results. HPV testing using hc2 technology is superior to DNA PCR testing.

Abbreviations used- HPV DNA PCR (Human papilloma virus, Deoxyribonueclic acid, Polymerase chain reaction.), hc 2(Hybrid capture 2),PAP smear (Papanicolaou smear), HSIL (High grade squamous intraepithelial lesion), LSIL (Low grade squamous intraepithelial lesion), CIN (Cervical intraepithelial Neoplasia, FOGSI(Federation Of Obstetricians & Gynaecological Socities of India).

MATERIAL & METHODS

Part 1-The present study was conducted in the Department of Obstetrics & Gynaecology. Study period for HPV DNA PCR from June 2009 to Dec 2010.

Inclusion criteria-

All included except as give below.

Exclusion criteria-

Individuals concurrently enrolled in clinical studies of investigational agents or studies involving collection of cervical / genital specimens.

History of vaccination with HPV vaccine.
Women who had undergone hysterectomy or treatment for cervical cancer in past.
Pregnancy
Active bleeding or infective discharge
Any condition which in opinion of investigator might interfere with evaluation of women enrolled for study.
Inability to give consent.

Volume : 6 | Issue : 3 | March 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Study conduct
Ethical committee sanction was taken for study. Written informed consent was taken was taken from patient. Women underwent PAP smear test. Women with abnormal PAP report underwent HPV DNA PCR testing.

Laboratory assessment
Endocervical scraping on cytobrush with sterile saline obtained and transported at room temperature. Sample processed and DNA subjected to amplification and reamplification by PCR. Detection HPV DNA in sample was done by using gel electrophoresis technique. Genotype identification was done by DNA PCR using MY09 and MY11 primers. HPV serotype was identified by direct nucleotide sequencing which is gold standard for identification of HPV subtypes. The PCR based assay along with nucleotide sequencing for HPV typing has specificity of 98-100%.The assay has high sensitivity with lower limit of detection being 1250 viral particles per mg of tissue.

Part 2 of study was conducted from January 2012 to 30 July 2012.

Same Inclusion & exclusion criteria were used.

Women who had abnormal PAP smear underwent Digene Hybrid capture technology (hc2- signal amplification of target DNA). Assay detection limit is the threshold ratio above which it is considered as positive for clinically significant HPV load. The analytical sensitivity of hc2 is 5,000 copies of HPC DNA.

Result interpretation is ratio (Relative light units / cut off) for HPV 16,18,,31,33,35,39,45,51,52,56,58,59& 68, i.e. 13 oncogenic high risk types.

All women who walked in Gynaecological outpatient department were counselled for PAP smear.

Women who had positive PAP report had cervical sample collected using DNA PAP Cervical sampler & processed for HPV DNA hc2test.

Results were interpreted & generated.
Patient's data were interpreted & analysed.

RESULTS
Part 1- Study with HPV DNA PCR-

PAP smears were done. 27 women had abnormal PAP report.

15 LSIL of which none had HPV DNA PCR test positive.

12 HSIL of which none had HPV DNA PCR test positive.

Part 2- Study with HPV DNA hc2. Surprisingly again 27 women had abnormal PAP report.

Out of 19 LSIL PAP report 4 women were positive for HPV DNA hc2 that is in 20% women.

Out of 8 HSIL PAP report 2 had HPV DNA hc2 that is in 20% women.

DISCUSSION
As per expectation there should have been higher positives as far as DNA PCR is concerned as these women already had HPV induced changes.

PCR picks even DNA count as low as 5 particles where as hc2 should be positive when 5000 DNA particles are available.

In patients of established cancer cervix both tests showed 100% positivity. Thus some changes are occurring during transformation from cervical intraepithelial lesion to cancerous change.

The Change is- during viral transformation L1 can get deleted. GP5+/ 6+ primers will miss disease. False negative is a possibility.

HPV genome is very well studied and when a mitotically active cell is infected with virus it will remain in cell in low numbers as low as 100 copies & can be detected by PCR only. This shedding will be intermittent as well.

The key alteration that takes place during integration of viral DNA in to host chromosome is that the circular DNA has to change to linear physical state.

As this PCR test used by us was using L1 probe resulting in failure to detect physically altered virus. (5)

Merits of hc2 test-
This technology detects both the early (E1-7) and late (capsid proteins) genes, making it more sensitive in detection of HPV. (4)

Search for improved HPV testing methods should continue to give us methods that catch viral DNA in action that have caused CIN 2 or3 changes than that just give information of their presence. (4)

REFERENCES
1. Jyotsna Potdar. To study Human Papilloma Serotype Prevalence in selected married sexually active women. Indian Journal Of Applied Research.Vol1, Issue1.Oct2011.
2. Jyotsna Potdar. Human Papilloma Testing In Midlife, just once!- Indian Journal of Applied research. Vol5. Issue 2. Feb2015.
3. Lorincz (Methods of HPV detection); emerging Issues of HPV infection. From science to practice, Monsonego 2006.
4. Jorda P. Reynold; HPV: A new look into new methods for high risk testing. Medical Lab Observer. July 2011.
5. Te Linde's Operative Gynecology. 10th Edition.pg 1212-1213.