

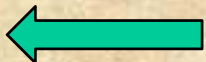
**Identification of Human *papillomavirus* Infection  
in the Pap Smear Samples Using  
Liquid Based Cytology and Polymerase Chain  
Reaction in Birjand**

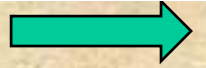
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# Introduction

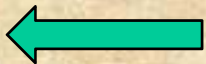
- *Human papilloma virus* (HPV) is an icosahedral DNA virus
- The Papillomaviridae family is a very large family divided into 16 genera, of which five contain members that infect humans (*Alpha-*, *Beta-*, *Gamma-*, *Mupa-*, and *Nupapapillomavirus*).
- To date, more than 200 types have been identified
- There are about 40 HPV viral types that are commonly found in the genital tract
- Human papillomavirus (HPV) infection is a sexually transmitted disease (STD) and the single most important etiological agent of cervical cancer.



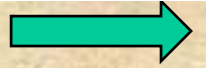


# Cervical cancer

- Worldwide, cervical cancer is the second most important cause of cancer-related mortality in women, after breast cancer.
- Cervical cancer can be easily diagnosed in its preinvasive stage
- pre-invasive cervical abnormalities persist for several years and can be effectively treated before it progresses to invasive cancer.







- Previous studies have shown that nearly 100% of cervical invasive squamous cell carcinomas are infected with high-risk oncogenic HPV types.
- Fortunately most HPV infections clear spontaneously, yet a minority of infections, especially high-risk infections, do persist.

## Different HPV detection methods exist

- Direct nucleic acid Probe Methods (Southern blot and In Situ Hybridization)
- Signal amplification methods (Digene's Hybrid Capture II)
- (DNA) target amplification methods, such as polymerase chain reaction (PCR), allow a more sensitive detection of viral DNA.
- Direct examination, cytology, Pap smear

## HPV low risk & high risk

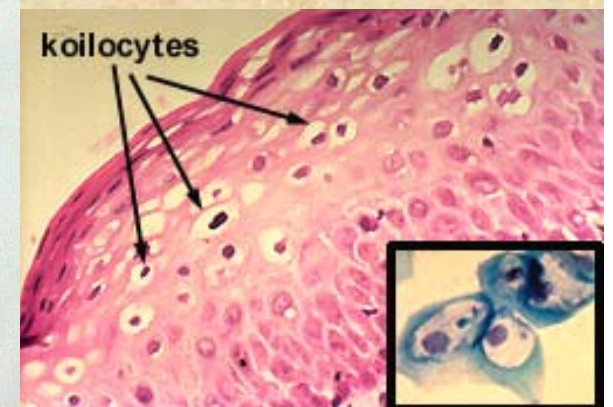
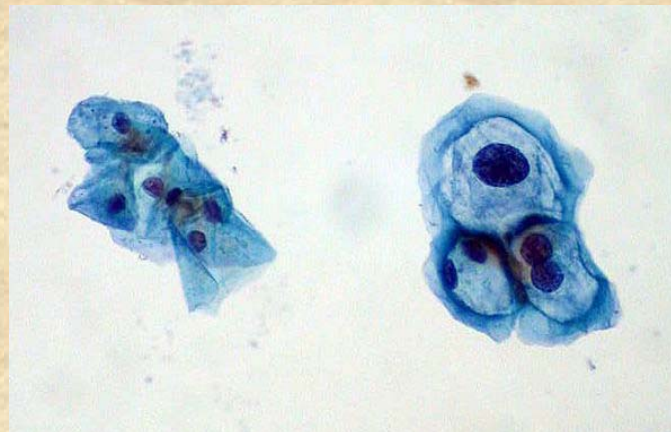
- HPV16 and HPV18. , 58 , 56 , 52 , 51 , 45 , 39 , 35 , 33 , 31 , 73 , 59 , 68 and 82 as high risk
- Types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 are accepted to be low-risk HPV (LRHPV),
- **Early detection** of oncogenic genotypes in women with abnormal Papanicolaou (PAP) smears may permit selection of patients who are at increased risk of progression to invasive cancer and therefore, can be of significant clinical value for further diagnostic and therapeutic approaches.



# Cellular changes associated with CIN and HPV

A **Koilocyte** is a squamous epithelial cell that has undergone a number of structural changes, which occur as a result of infection of the cell by human *papillomavirus*:

- *Nuclear enlargement (two to three times normal size)*
- *Irregularity of the nuclear membrane*
- *A darker than normal staining pattern in the nucleus, known as Hyperchromasia*
- *A clear area around the nucleus, known as a perinuclear halo.*



## Population of study

- An average of 130 genital specimen was taken from women with genital inflammation from October 2013 to April 2014
- These include smears classified as atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), low-grade squamous intra-epithelial lesion (LSIL), high-grade intra-epithelial lesion (HSIL) and invasive carcinoma.
- All patients provided informed consent for smear taking and HPV testing. The study was approved by the Birjand University of Medical Sciences Ethical Committee.



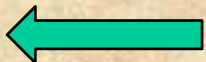
# Laboratory assays

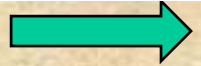
- **Cytology:**

Epithelial cells from the cervix were collected with the Cervex-Brush (Rovers, Oss, The Netherlands), suspended immediately in an ethanol-based preservative fluid.

- **Pap test** (papanicola staining with thin layer)

- The remaining cell suspension of the abnormal samples was forwarded to the laboratory for molecular diagnostics.





- **HPV DNA extraction:**

- ✓ After defrosting the sample, 200 microliters subjected to DNA isolation with Bioneer DNA extraction kit, south Korea.
- ✓ cellular Beta globins gene PCR was used for control of extraction.

- **HPV DNA detection:**

- ✓ A PCR test was performed with a set of primer (MY09 – MY11) amplifying 500 bp of L1 gene, detecting all types.
- ✓ HeLa cell line was used for +tive ctr and water for –tive.

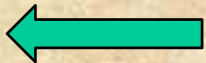
## results

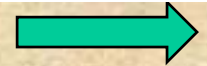
- the mean age of patients was 31/67 years.
- Cytopathology testing showed that only 3 % had an abnormal cytology(CIN++), with a predominance of atypical squamous cell of undetermined significance cases.
- Direct examination showed that 23 cases (17.7 %) were positive for HPV (koilocyte positive) , from which 22 cases (95.65 %) had also HPV DNA.
- Moreover 7 other cases of negative cytology result had HPV- DNA, 6.5% of normal cytology.
- overall 22.3 % were PCR positive.



## Discussion

- Although testing for HPV has higher sensitivity and lower specificity than cytology alone for detecting cervical intraepithelial neoplasia (CIN), studies comparing conventional and liquid-based cytology have had conflicting results.
- This project showed that all cytology HPV+ had HPV DNA, more ever 7 other cases of negative cytology cases had HPV- DNA.
- 6.5% of normal cytology cases were HPV+ with PCR test.
- In a survey by Kashyap, et al 38% of whom with normal cytology had HPV- DNA.





- And also *Guglielmo Ronco et al* showed that HPV testing had a more sensitivity than cytology alone (relative sensitivity = 1.47; 95% confidence interval [CI] = 1.03 to 2.09).
- Pontus Naucler also found similar result in a 35% (95% confidence interval [CI] = 15% to 60%) increase in sensitivity to detect CIN3+, without a statistically significant reduction in the PPV (relative PPV = 0.76, 95% CI = 0.52 to 1.10),

## Conclusion

- Based on these data, a combination of cytology and HPV DNA testing allows for identification of patients with HPV infection and improves cervical cancer prevention.
- It is suggested that high-risk HPV detection can be utilized as an adjunct to routine cytology screening programs to identify 'high risk' women who have concurrently negative Pap smears but may harbor oncogenic HPV infection and/or more likely to develop CIN lesions.



**Thanks of your attention**

