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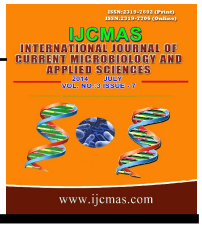

Original Research Article

Estimation of Human Papilloma virus DNA in Urine Specimens as a marker for Cervical Cancer at an early stage

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Keywords

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A B S T R A C T

To investigate the usefulness of human papillomavirus detection in the urine of women with poor gynecologic attention. Materials and Methods. 1000 urine and 1000 cervical samples from 1000 women were analyzed. Polymerase chain reaction was performed on these 2000 samples using biotools kit for HPV detection. The concordance of the results between both sample groups was 80%. In the urine samples, the sensitivity of polymerase chain reaction for high-grade squamous intraepithelial lesion was 100%, the specificity was 80%. Human papillomavirus detection in urine samples may be used as an alternative screening method for women with poor gynecologic attention and the invasive method for HPV detection can be avoided.

Introduction

Cervical cancer is global public health problem and most common cancer in women in developing countries. The burden of cervical cancer in India is enormous accounting for about 20% of all cancers related deaths in women and is the number one cause of death in middle aged Indian women. Cervical cancer is caused by "high-risk" types of human papillomavirus (HPV) sexually a common transmitted infection. Most cervical cancers are curable and can be prevented by regular screening with hybrid capture testing(pap test and HPV DNA test).Regular screening reduces morbidity and mortality of women by cervical cancer.

In developing countries lack of effective screening programs for cervical cancer has resulted in no clinically significant reduction in the incidence of cervical cancer during the past three decades. By contrast in developed countries, there has been a major decline in cervical-cancer mortality after the introduction of large-scale screening procedures .Cervical cancer screening can lead to 70% reduction in mortality, still it is not in top ten national health priorities in India. Preliminary studies have revealed negligible awareness in the selected community. In the study population no such

awareness generating and screening programme for cervical cancer has ever been carried out before. The aim of our present study was to check the diagnostic efficacy of human papilloma virus (HPV DNA) testing in cervical scrapings and urine for detecting cervical lesions was compared. Noninvasive screening procedure could be firmly established which would help overcome major obstacles in effective cervical cancer screening and serve as a prototype for such screening programmes in future.

Human papilloma virus (HPV) is the cause of most common sexually transmitted diseases (STDs) of viral etiology worldwide .High-risk HPV types, HPV 16, 18, 31, and 45, are more closely associated with anogenital malignancies and have been implicated in the etiology of most, if not all, cervical cancers. Cervical cancer is the second most common cancer of women worldwide. It has very good prognosis detected at an early stage. Regular screening is thus very important .As per recommendations of American College of Obstetricians and Gynecologists(ACOG) and American Cancer Society(ACS) guidelines women thirty years or older be offered a triple test: HPV DNA test in conjugation with pap smear and pelvic exam.

Although Papanicolaou (Pap) smear is currently a standard for cervical cancer screening it has certain limitations. Only 15 to 50% of patients with HPV infections are accurately identified by Pap smears [1,2]. Repeat visits are needed. Further, it has been observed that it is very difficult to motivate Indian women for voluntary pelvic examination and pap smear. Pelvic examination is invasive and uncomfortable for the patient and time-consuming for the health care provider [3]. Human papillomavirus (HPV) detection is useful as a screening method for high-grade cervical (CV) lesions and CV cancer in the general population, especially in high-risk groups. Human papillomavirus detection can be performed classically in CV cytological or biopsy samples which are invasive and needs intervention of health care provider.

Purpose of present study was to evaluate and establish use of urinary HPV-DNA testing a mass screening tool, for cervical cancer. In developing countries where gynecological attention is poorer than in developed countries, it has been observed is need based and practical to analyze self collected samples rather than those collected by trained professionals- especially while screening for various disease conditions. Urine samples can be self collected or easily collected by volunteers /social workers. to study the possibility of applying this detection method to patients with poor gynecologic attention. In this population based study 1000 women were motivated for pap smear and HPV DNA testing and their urine and cervical swab was collected. PCR was done in 2000 samples and concordance was recorded between cervical swab and urine samples which was found to be 80%.

Materials and Methods

Samples

1000 women through camps and various hospitals were motivated for pap smear and their cervical swabs and urine samples were collected. The study included 1000 patients with urine samples and matching cervical swab samples. Although the specimen collection sequence was not strictly enforced, the urine was usually collected prior to the pelvic examination. All study participants signed informed consents, and the study was approved by the Institutional ethical committee, Government Medical College Srinagar. Demographic data were collected by questionnaire.

Collection of Cervical swabs

One cervical swab specimen was collected from each patient by use of a cervical spatula, the swab was then placed in 1 ml of normal saline solution and stored at −20°C until use.

Extraction of DNA from cervical swabs

Sample extraction was based on the protocol given by biotools kit. The DNA extracted by kit method was stored at −20°C until use.

Extraction of DNA from urine

All urine specimens collected were stored at 4°C for a minimum of 24 h. 12 milliliters of each urine sample was centrifuged at 4, 5000 × *g* for 15 min at room temperature. Further protocol was followed as given by biotools DNA extraction kit and the DNA extracted was stored at -20°C until use.

PCR

Urine and matching cervical swab specimens were subjected to PCR for amplification of HPV by using biotools kit for PCR (Containing HPV mixture, $MgCl_2$, Taq polymerase, Positive control). The PCR product was analyzed in 3% agarose gel and was visualized by Gel Documentation system.

Results and Discussion

The study population consisted of 1000 women who donated urine specimens and matching cervical swab specimens. The mean age of the patients was 35 years, ranging from 26 to 52 years. 300 (30%) cases were diagnosed as atypical squamous cells of undetermined significance (ASCUS), 220 (22%) cases were diagnosed as low-grade squamous intraepithelial lesions (LSILs), and 67 (6.7%) cases were diagnosed as high-grade squamous intraepithelial lesions (HSILs). The results of the HPV are analysed. A positive result was found in 240 (24%) of 1000 CV samples and in 196 (19%) of 1000 urine samples. Thus a concordant result between both types of samples was obtained in around 80% . In total 760 were negative and 240 were positive for HPV analysis in CV samples but 804 were negative and 196 were positive for HPV analysis in urine .All cases positive in urine specimens for HPV DNA were positive in CV specimens . All HPV-negative cases in both urine and CV samples were ASCUS or LSIL. No cases diagnosed as LSIL were found to be negative in the CV sample and positive in the urine sample. However only 02 cases diagnosed as HSIL were negative in the CV samples and urine samples. Of the 240 cases positive in the CV samples 175 were LSIL or ASCUS and 65 were HSIL. Of 196 cases positive in both the CV and urine samples 65 were HSIL, and only 131cases were LSIL or ASCUS.

In this study analyzed the usefulness of HPV detection in urine samples compared with CV samples. The concordance obtained in our study between CV and urine samples was 80%. This demonstrates that detection of HPV DNA in cervical swab specimen is higher than that from a urine specimen. As far as HSIL is concerned whether the sample is CV or urine there is 100% concordance in cases with positive HPV-DNA .The difference in HPV DNA detection in CV or urine samples is only in cases which are diagnosed LSIL or ASCUS. 340 (34%) of the 1000 cases studied were positive for HPV in the CV samples, and 220 (22%) of these 1000 cases were positive in the urine samples. The positivity reported on the literature ranged from 40% to 90% in CV samples and from 15% to 75% in urine samples [4, 5]. Other authors reported a higher HPV positivity of 98% in CV samples and 71% in urine samples, but all

the cases included in this study were infiltrating squamous cell carcinomas [6]. The positivity in urine samples was always lower than the positivity in CV samples. The lower detection rate of HPV positivity in our study (34%) can be because only 28% of our cases were HSIL and 40% of the samples analyzed were ASCUS. None of the ASCUS cases analyzed in our study was positive for HPV in the CV or in the urine samples, although the reported HPV positivity in these lesions in CV samples are around 20% to 40% [7]. Some authors have reported HPV in 15% of the urine samples of patients with a normal Pap test [6]. 10 of the LSIL cases showed positivity for HPV, all of them in CV samples but only 2 of them in the urine sample. Furthermore, all of the HSIL cases were positive when considering either the CV samples or the urine samples, or both. 4 HSIL cases were only positive in the urine samples; and in both cases, high-risk HPV (16 and 31 HPV) were detected. 16 of 28 HSIL cases were positive in both samples, and 8 of 28 HSIL cases were positive only in the CV samples. Consequently, HPV detection in the urine samples showed a high sensitivity for the detection of the HSIL cases and also a high specificity because 20 of 22 HPV-positive cases in the urine samples were HSIL and only 2 were LSIL. We can therefore conclude that HPV detection in urine samples may be used as an alternative screening method because HPV detection in urine samples shows a high sensitivity and specificity for HSIL.

In summary, we have developed a urine-based assay for detection of the DNA of HPV, which is responsible for nearly all cases of cervical cancer. The assay with urine appears to be as adept as the assay with a cervical swab specimen for the detection of any HPV type or any high-risk HPV type. Given that instances of discordant results for HPV DNA detection between the urine specimens and the cervical swab specimens may potentially be rectified by testing multiple urine specimens from the same patient. Also may be of little significance as we are missing only ASCUS /LSIL Cases.

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References

1. Meisels, A. 1983. The story of a cell. The George N. Papanicolaou Award lecture. Acta Cytol. 27:584-596.
2. Purola, E., et al. 1977. Cytology of gynecologic condyloma acuminatum. Acta Cytol. 21:26-31.
3. Reddy, et al. 1997. Patient anxiety during gynecologic examinations. Behavioral indicators. J. Reprod. Med. 42:631-636.
4. Strauss S, et al. Detection and typing of human papillomavirus DNA in a paired urine and cervical scrapes. Eur J Epidemiol 1999;15:537Y43.
5. Jacobson DL, et al. Concordance of human papillomavirus in the cervix and urine among inner city adolescents.Pediatr Infect Dis J 2000;19:722Y8
6. Brinkman JA, et al. Detection of human papillomavirus DNA in urine specimens from human immunodeficiency virusYpositive women. J Clin Microbiol 2002;40: 3155Y61.
7. Solomon , et al. The Bethesda System for Reporting Cervical Cytology, 2nd ed. New York: Springer Verlag, 2004.