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Diagnosis of cervical cancer by a urine test

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ABSTRACT:
Cervical cancer is the fourth most common malignant tumor among women in the world. However, 90% of deaths occur in developing countries. Tumor pathogenesis is associated with exposure to high-risk human papillomavirus (hrHPV), most often 16 and 18 strains. The sooner precancerous lesions or cancer are detected, the higher the chance of survival is. That is why prophylaxis is so important in this case. Due to the low turnout of women in cytology, new, alternative methods of prevention are needed. According to the research, women prefer tests in which samples are taken by themselves. Hence, more and more studies on the use of urine in the prevention of cervical cancer. Urine is a material that is easy to pick up. Patients feel comfortable because they can do it by themselves. Still, more research is needed to optimize its collection, transport, or tests used on samples.

KEY WORDS: human papillomavirus, uterine cervical neoplasms, urine

INTRODUCTION:

Cervical cancer is the primary malignant tumor of this organ. The pathogenesis of cancer and precancerous lesions, i.e. CIN (cervical intraepithelial neoplasia), is associated in most cases with exposure to high-pathogenic HPV (human papillomavirus) - 16 and 18 (account for 70% of cases of disease [1]), and strains 31, 33, 45, 52 and 58 [2]. Risk factors of this cancer are: early age of sexual life, numerous sexual partners, smoking, pregnancy and childbirth at an early age, and the occurrence of this cancer in the family [3]. HPV viruses are characterized by tropism to immature epithelial cells of the transitional cervix. Infected cells express oncoproteins. E6 and E7 oncoproteins bind and inactivate p53 and Rb suppressor genes, which promotes carcinogenesis [4]. The most common histological types of cervical cancer are squamous cell carcinoma and adenocarcinoma. The peak of illness occurs around the age of 45 and the symptoms reported by the patient are non-specific. These include unexpected bleedings, vaginal discharge, painful urination and sexual intercourse. The tumor infiltrates the surrounding tissues, and also passes through the lymphatic vessels to the lymph nodes or via the blood vessels to the lungs and bones. In order to assess the staging of this cancer, we use the four-stage FIGO scale, where:

- I is carcinoma limited to the cervix,
- II carcinoma extends beyond the cervix and/or succumbing, but does not infiltrate the lower third of the vagina or pelvic wall,
- III - cancer infiltrates the lower third vaginal walls and pelvic wall,
- IV-cancer infiltrates the bladder, rectum and gives distant metastases [5].

Cytological examination is an example of cervical cancer prevention. It involves scraping the epithelial cells of the cervical transition zone, and then evaluating the smear according to the Bethesda scale. This allows for on early detection of pre-cancer lesions. If an abnormal cytological result is obtained, the changes or their absence may be verified by histopathological examination. However, the consistency of samples varies from 40% to 89% depending on the literature, which indicates the possible wrong results of this method [6]. Another method is the detection of high-pathogenic HPV strains in cervical scrapings.

According to the WHO, cervical cancer is the fourth most common malignant tumor in women. In 2018, about 570000 new cases were diagnosed, which accounted for 6.6% of malignant tumors of women. 90% of the deaths caused by this cancer have been reported in developing countries. The earlier the cancer is detected, the higher the chance of being cured. The mortality rate may be reduced by appropriate education, prevention or early diagnosis [7].

The aim of this work is to present new, effective, non-invasive methods to diagnose cervical cancer by means of a urine test. Urine test can increase the comfort of women undergoing testing. It can allow them to gather samples for the test alone, which can affect a larger number of women undergoing prevention of this cancer and thus may reduce the mortality rate.

A REVIEW OF AVAILABLE RESEARCH:

Research conducted in the Federated States of Micronesia showed that out of 217 women, up to 95% were satisfied with the urine test. In the case of cytology it was 82% of the patients, but only 42% of women would prefer that an experienced clinician doing it. These studies suggest that self-sampling is preferred among women [8].

In the case of a study conducted in Korea, 732 women aged 20 to 69 showed that overall satisfaction was significantly higher for both vaginal sampling and urine sampling compared to the cytology performed by the clinician (odds ratio [OR] = 2.01, 95% confidence interval [CI] = 1.48-3.00 and OR = 2.47, 95% CI = 1.75-3.48, respectively). This suggests that the possibility of self-sampling may increase the number of women undergoing cervical cancer prevention [9].

Research in Thailand has shown that attendance in screening tests for cervical cancer detection is very low (25-38% of women aged 30-35 have had a cytology performed once in life). In a study of 164 women, cervical swabs and urine samples were compared using HPV test (HPV Geno Array Diagnostic Kits). The overall agreement between paired samples was 62.5%. Analysis of urine samples and a second analysis of cervical smear samples showed that differences in the overall rate of HPV detection between women with normal and abnormal cytology were not significant ($p > 0.05$). This result suggests that urine is a feasible and possible substitute for cervical smears. Urine test to

detect infection with high-pathogenic HPV strain may be an alternative to cytology [10]. 240 women took a urine sample themselves, and the clinician additionally performed a cytology. Among all examined samples, the incidence of HPV was 42.9% among urine samples. The compatibility between the two types of samples was 98.4%, k = 0.792. Incompatible results were observed in 27 cases; 5 were positive only in urine samples, and 22 were positive only in swab specimens. The sensitivity and specificity for total HPV DNA in the urine fraction using cervical samples as reference was 68.4% and 99.9%. The results of these studies also suggest that urine may be a non-invasive, alternative method for detecting HPV infections [11].
Studies in Spain consisted of comparing samples of urine collected in the morning, urine collected later, material taken independently from the cervix, and material collected by the clinician. Samples from 91 patients were analyzed. All 6 cases of CIN3 showed a positive hrHPV test in each type of sample, in both HPV tests (SPF10-DEIA-LiPA25 and GP5+/6+-EIA-LMNX). The sensitivity for CIN2+ in the SPF10 system was 95% for the urine sample collected in the morning and 100% for all other samples. In the GP5+/6+ test, the sensitivity was 95% in all types of samples. The sensitivity and specificity for both tests on each type of samples did not differ significantly. There was a 10-14% inconsistency in the hrHPV genotype. Similar sensitivity of CIN2+ was shown for HPV testing in the first-void urine, a smear taken by a doctor and a cervical sample taken alone [12].
Studies in Belgium on 110 women from 25 to 64 years of age directed to colposcopy suggest that first-void urine samples may be an alternative to cervical specimens to detect HPV DNA. In the case of high-risk HPV strains, the compliance of paired urine samples and cervical smear was very high (Kappa Cohen 0.688 (95% CI: 0.542-0.835)). In addition, women have been shown to prefer self-collection of urine to the study than the cytology performed by the clinician [13].
In North Thailand, studies have been carried out on HPV+ women. Urine and cervical samples were obtained from 168 women. Out of 123 correctly collected paired samples, compliance in high-risk HPV DNA detection was present in 106 cases (86.2%), with kappa statistics of 0.65 (significant compliance). Using the HPV results from the cervix as a reference, the sensitivity of HPV tests in the urine was 68.6% (24/35) and the specificity was 93.2% (82/88). In order to detect HSIL+, the sensitivity of HPV in the urine was 80.0% (4/5) and the specificity was 78.0% (92/118). HPV in urine had a high specificity in HPV detection, as well as high sensitivity in histological detection of HSIL+ [14].
Tests carried out in Korea consisted of taking vaginal and urine samples by the patient herself. High-risk HPV strains were detected in 6.7% of urine samples and in 9.6% of vaginal smear specimens. HPV 16/18 was detected in 1.5% (other hrHPV strains 5.2%) of urine samples and 2.0% (other hrHPVstrains 7.6%) of vaginal smear samples. Although a statistically significant difference in the frequency of hrHPV detection between urine samples and vaginal smears was observed ($p < 0.001$), the compliance for HPV 16/18 was relatively high (99.1%, 95% CI 98.1 ~ 99.6%), from kappa 0.75. In addition, satisfaction with self-collection of both urine samples (91.4%) and vaginal swabs (92.7%) was higher than in the case of the clinician (88.1%). The study suggests that self-sampling may be an alternative to the clinician's performance [15].
In the case of detection of hrHPV infection in a woman, this result can be confirmed by the presence of DNA methylation markers in the cervical material. It was decided to check whether these markers are also detectable in urine samples. 43 urine samples and 38 paired cervical scrapings were collected from patients with cervical cancer, aged from 27 to 86 years. It has been shown that both native urine (24/28-86%) and sediment (25/28-89%) are suitable for the detection of high-risk HPV strains as well as DNA methylation markers. A strong relationship was found, both between native urine and sediment and all methylation markers tested (FAM19A4, GHSR, PHACTR3, PRDM14, SST, ZIC1). The results of the test for the presence of HPV and methylation markers in the urine and in the cervical scrapings were compared. In paired samples, hrHPV infection was detected in 31 (82%) urine sediments and 34 (89%) of cervical scratches, what led to almost perfect compliance (with kappa value of 0.85, which gives a 95% confidence interval) [16].
In the case of CIN2+ detection studies, the preservative fixed urine showed good compatibility with the vaginal samples to detect hrHPV. The detection sensitivity of CIN2+ was 15/18 (83%) for urine and 16/18 (89%) for cervical and vaginal samples according to ART (Abbott RealTime), and 15/17 (88%) for all samples by RC (Roche Cobas 4800). Urine tests have been shown to be widely accepted by women. It suggests that research should be continued to develop an alternative method of

prophylaxis [17].
For detection of HPV DNA in the urine was accepted as a screening test for cervical cancer, there is research on the most effective method. Innovative studies were conducted involving the use of polypyrrole polyolefin polypeptides (PEI-mPpy NW) coupled to polyethyleneimine for the extraction, identification and detection of colorimetric strains of HPV DNA in urine samples of patients with cervical cancer. A 100% compliance rate was obtained between urine samples and cervical smears, even with a small amount of urine (300 μL). This method gives high hopes and may be a future in the prevention of this cancer [18].
Studies conducted on 43 patients in Puerto Rico showed that there are three metabolites in the urine of women infected with high-pathogenic HPV strain: 5-oxoprolinate, erytronic acid and N-acetylaspartic acid. All of them differentiate samples from negative samples as well as samples infected simultaneously with high and low-oxygen HPV strain. However, it is necessary to study a larger group of patients to prove this finding [19].
Another study shows that during cervical uterine cancer, the concentration of 60 different proteins increased, including leucine-rich α-2-glycoprotein (LRG1) and isoform-1 multimerin-1 (MMRN1). In contrast, the concentration of 73 proteins decreased, such as the S100 A8 (S100A8) calcium binding protein, serpin B3 (SERPINB3) and the differential antigen-44 cluster (CD44). ROC analysis showed that LRG1 and SERPINB3 can be used individually to detect cervical cancer. It was also shown that these 5 proteins together can be used in the diagnosis of this cancer [20].

CONCLUSIONS:

Urine testing may be the future and an alternative to other screening methods in the diagnosis of cervical cancer. This may increase the number of women undergoing screening due to the greater comfort, ease and ability to perform the test by themselves. Faster detection of pre-cancerous and cancerous lesions will increase the chance of recovery and also the survival rate. However, further research is needed to optimize this method such as how to retrieve material for test, how to store it, and how to research it.

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