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Application of molecular genotyping to determine prevalence of HPV strains in Pap smears of Kazakhstan women

Luiza Niyazmetova [a], Gulzhanat Aimagambetova [a], Nazigul Stambekova [b], Zaurech Abugalieva [c], Korlukain Seksembayeva [c], Syed Ali [a], Azliyati Azizan [a,*]

[a] Nazarbayev University School of Medicine (NUSOM), 53 Kabanbay Batyr Ave., Astana, 010000, Kazakhstan
[b] University Medical Center, Mother and Child Hospital, 32,Turan Avenue, Astana, Kazakhstan
[c] University Medical Center, Republican Diagnostic Center, 2, Syghanaq Street, Astana, Kazakhstan

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ABSTRACT

Objectives: Human papillomavirus is the main causative agent for cervical cancer. However, few data are available about HPV prevalence in Kazakhstan. The aims of this study were to genotype HPV DNA in Pap smear samples of women to determine prevalence of carcinogenic HPV types in Astana, Kazakhstan and to analyze the association between HPV positivity and the cytology results of patient samples.
Methods: Pap smear materials were obtained from 140 patients aged 18-59, who visited the outpatient gynecological clinic. Microscopic examination was done to detect dysplasia, and HPV genotyping was done using real-time multiplex PCR.
Results: HPV testing showed that among 61 HPV positive patients, the most prevalent types were 16 and 18. Microscopic examination showed that 79% of the samples had normal cytology, while 13% had CIN grade I, 5% had CIN grade II, and 3% had CIN grade III. The analysis revealed that 12% of the samples had CIN cytology and presence of HPV. Approximately 31% had HPV without cervical dysplasia, while 8% of samples were CIN positive without HPV infection. A statistically significant relationship between HPV 16 and HPV 33 positive samples and CIN grade II and III was found.
Conclusions: Overall, this study will help to strengthen and guide health policy implementation of primary and secondary cervical cancer prevention strategies in Kazakhstan.

1. Background

Cervical cancer is the third most diagnosed type of cancer and the fourth leading cause of death in women worldwide[1]. Approximately 99% of all cervical cancer cases have been linked to Human papillomavirus (HPV) infection, which has also been associated with other anogenital cancers (anus, vulva, vagina and penis), and head and neck cancers[2]. HPV is a small non enveloped double-stranded DNA virus of Papillomaviridae family[3]. Of the more than 100 HPV types, 15 (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) were identified as high risk (HR) due to their strong correlation to cancers of the anogenital region[4]. HPV types 16 and 18 are responsible for about 70% of all cervical cancer cases worldwide, while other HPV types like 31, 33, 35, 45, 52 and 58 account for 20% of cervical cancers in the world[5]. Screening for cervical cancer with the Papanicolaou cytology technique, or Pap smear test for short, has become widely accepted. The Pap smear test is aimed to detect cervical intraepithelial neoplasia (CIN), which is a premalignant transformation in the cervix. Additionally, HPV DNA testing for HR-HPV types is also recommended to improve the accuracy of screening for cervical cancer, and to increase chances for recovery[6].

Current estimates for Kazakhstan indicate that every year 2,789 women are diagnosed with cervical cancer and 982 die from the disease[2]. Cervical cancer in Kazakhstan ranks as the second most frequent cancer among women and the first most frequent cancer among women between 15 and 44 years of age with the incidence of 32.8[2]. However, there is still lack of sufficient information about the prevalence of HR-HPV types in Kazakhstan. Having more information about the types of HPV strains that are prevalent in patients with abnormal cytology and hence are at high risk for developing cervical cancer can provide guidance regarding preventative measures against cervical cancer. This pilot study

* Corresponding author. Nazarbayev University School of Medicine (NUSOM), 53 KabanbayBatyr Ave., Astana, Republic of Kazakhstan, 010000.
E-mail address: azliyati.azizan@nu.edu.kz (A. Azizan).

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on HPV in Kazakhstan was undertaken with two main objectives; the primary objective was to determine the most prevalent HPV types in Astana, Kazakhstan by genotyping HPV DNA in Pap smear samples of women that are at risk to develop cervical cancer. The second objective of this study was to analyze the association between HPV positivity and the cytology results of patient samples. This also serves as a feasibility study that will form the basis for a larger study. Given the high incidence of cervical and other HPV associated cancers in Kazakhstan and the Central Asian countries, findings from such studies could contribute toward informing relevant policies related to screening and preventative vaccination and other public health HPV programs in the region.

2. Methods

2.1. Study population and sample collection

The total number of patients in this study was 140 women of ages 18 – 59 years. The samples were collected as part of a screening procedure, called Pap smear, from all patients who attended the gynaecologist's office at University Medical Center (UMC), Astana, in the period from December, 2015 to April, 2016. The samples were collected in two sets in order to do microscopic analysis for abnormal cytology and for HPV DNA genotyping. The sample collection and analysis (microscopy and RT-PCR) were done on the same day.

2.2. Ethical consideration

The Nazarbayev University Ethical Committee (IREC) granted exemption from IREC review for this study. Patient confidentiality was maintained whereby specific patient information (including name, contact and other sensitive personal information) was only accessible to the clinicians, and samples were number-letter coded to the investigators involved to conduct the protocols proposed for the study. Patient confidentiality was maintained throughout, and after completion of the study.

2.3. HPV DNA genotyping and cytological examination of samples

For HPV genotyping, real time multiplex PCR methodology using the specific PCR kit were used, following manufacturer's instructions (https://vector-best.ru/en/publ/list/maket_hpv.pdf - www.vector-best.ru). The laboratory method is based on simultaneous real-time multiplex PCR of HPV-specific DNA fragments and a noncompetitive internal control. The real time PCR instrumentation used for the assay is the CFX 96 Real -Time PCR (BIO-RAD). Microscopic examination of Pap smear samples was also performed using standard UMC hospital protocols to reveal cytological abnormalities[7].

2.4. Statistical analysis

All statistical analysis for this study was done with STATA 13.0 software. Simple statistical analysis such as determination of means, standard deviation, p-value, and odds ratio, was done for descriptive purposes. The association between age, HPV positivity status, and cytological pathology was analyzed with logistic regression.

3. Results

3.1. Prevalence of HPV types among positive samples

Twelve HPVs, which are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, genotypes were detected in our study samples using real-time PCR. Out of 140 samples analyzed by HPV genotyping, HPV DNA could be detected in 61 of samples (43.6% of total), and all 12 HPV genotypes could be identified collectively among these HPV positive samples. HPV DNA genotyping with real-time PCR showed that among 61 HPV positive samples, the most prevalent types detected were HPV 16 (18.4%) and HPV 18 (9.22%), followed by HPV types 33, 51 and 52 (nearly 5% each) (Figure 1). HPV types 59, 39, 31, 45, and 58 were found in at least 2% of the total amount of samples, while only about 1% or less of positive samples had HPV types 35 and 56.

3.2. Variation of cytology results among different age groups

The results of cytological examination and of HPV DNA genotyping with age distribution of patients are shown in Table 1. The age range of patients was from 18 to 59 years old, with a mean age of 33.5 ± 9.51 years (95% confidence interval (CI) 31.9 – 35.1 years). Among 140 cytology samples, 28 were identified as CIN grade 1, 2 or 3. The age group of 26-36 years had the highest number of HPV and CIN positive patients (Table 1). Regression analysis did not show statistically significant association between age and HPV positive status (p = 0.290). The results of Pap smear test

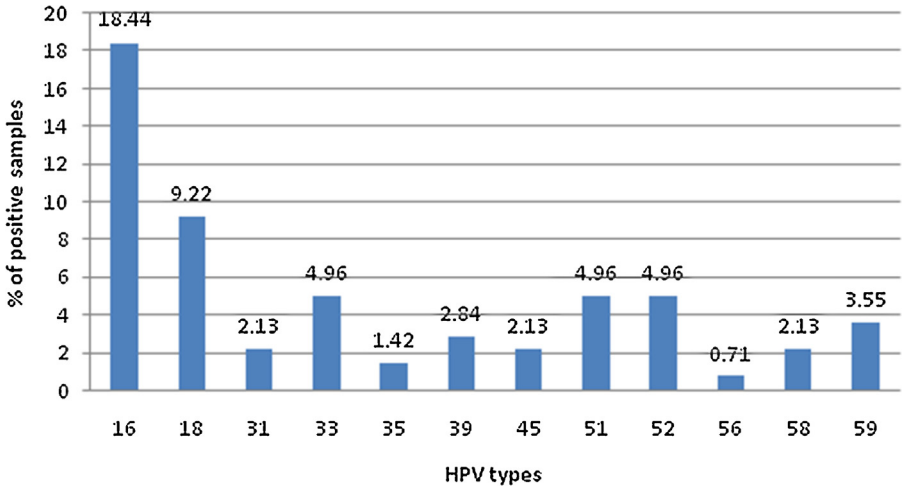

Figure 1. Distribution of HPV types among 61 HR-HPV positive patients, tested in Astana, Kazakhstan
Abbreviations
HPV = Human papillomavirus; HR-HPV = high risk HPV types.