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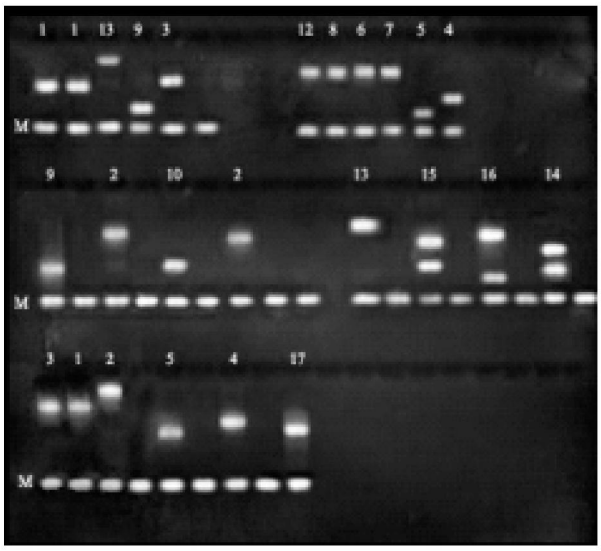
UROGENITAL INFECTIONS INFLUENCE ON CERVICAL EPITHELIUM PATHOLOGIES DEVELOPMENT IN CHILDBEARING AGE WOMEN INHABITING KAZAKHSTAN

The aim of the present study was to investigate urogenital infections in association with lesions at cervix in Kazakhstan women population. Cervical cells were collected from 1,000 women having different cervical pathology for detection the infectious agents using PCR assay kit. The Kazakhstan women population research showed presence of different types of bacterial, viral and protozoa agents in cases of inflammation and erosion processes in cervical epithelium. In pap smears diagnosed as heavy dysplasia, cancer *in situ* and invasive cancer only viral agents (HPV and HSV) were detected. HPV infection is not the main risk factor for cervical cancer development in Kazakhstan women population.

Introduction. Preneoplastic cervical epithelium abnormalities and cervical cancer are common factors of infertility between childbearing age women. Becoming pregnant after treatment for cervical cancer is highly improbable because one of the most common forms of treatment is a hysterectomy. Radiation therapy is another common choice but it can also damage reproductive organs thus making conception almost impossible. The situation worsens by the fact that cervical cancer is the leading cancer among women in many developing countries. In Kazakhstan about 1,000 women are diagnosed with cervical cancer each year and about 650 women die each year from this disease. Therefore investigation of cervical cancer etiology for the purpose of prevention and early recognition assumes ever greater importance. This kind of cancer is almost associated with infection by human papillomavirus (HPV). Nevertheless other genital microorganisms may be involved in the progression of HPV associated with lesions at cervix.

In this study HPV and other genital co-infections were investigated as possible factors promoting cervical cancer development risk in women population of Kazakhstan.



PCR diagnostics of urogenital infections: 1 - *Chlamydia trachomatis*; 2 - *Ureaplasma urealiticum*; **3** - *Gardnerella vaginalis*; 4 - *Mycoplasma hominis*; 5 - *Mycoplasma genitalium*; 6 - *Neisseria gonorrhoeae sicca, mucosa*; 7 - *Neisseria gonorrhoeae flava, subflava*; 8 - *Neisseria gonorrhoeae 1-3*; 9 - *Trihomonas vaginalis*; 10 -*Treponema pallidum*; 11 - *Herpes simplex virus (HSV I; II)*; 12 - *Herpes 6*; 13 -*Citomegalovirus*; 14 - *Pappillomavirus 6-11*; 15 - *Pappillomavirus 16-18*; 16 -*Pappillomavirus 31-33*; 17 - *Pappillomavirus 18*; M – internal positive control

Materials and methods

Study Population, clinics and cytology. Urogenital infections were investigated in 1,000 women including healthy and women having different cervical pathology. The mean ages of patients and controls were 32 years (15-49 years old). Cervical samples were taken with cytobrash from ectocervix and endocervix for further cytological verification of clinical diagnosis and detection of infections. Cytological probes (cervical smears) were divided into 5 groups: inflammation processes - 200, erosion - 200, severe dysplasia and cancer *in situ* - 225, invasive cancer - 225, normal epithelium using as a control – 150 cases.

PCR - detection of microorganisms. Low molecular weight DNA probes were extracted using DNA\RNA purification kit («***DNA-Technology***», Russian). Infections were detected by testing purificated DNA probes using PCR assay kit

(«***DNA-Technology***», Russian). PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining (Fig.).

Results and discussion

PCR analysis showed that in all studied groups *Chlamydia trachomatis* was present in 18% of the samples, *Ureaplasma urealiticum* - 48%, *Mycoplasma hominis* - *32%, Mycoplasma genitalium* - 25%, *Gardenella vaginalis* - 26%, *Trihomonas vaginalis* - 16%, *Treponema pallidum* - 5%, *Neisseria gonorrhoeae 1-3, flava, subflava, sicca, mucosa* - 30%, *Herpes simplex virus (*HSV *I,II,6,8,5-Citomegalovirus)* - 24%, *Pappillomavirus (6-11, 16-18, 31-33)* - 29%. The distribution of these urogenital infectious agents in smears reflecting different cervical pathologies is presented in table 1.

Analysis revealed the association between *Chlamydia trachomatis*, *Ureaplasma urealiticum, Mycoplasma hominis* and *genitalium, Gardenella vaginalis*, *Trihomonas vaginalis* and development of vulvovaginitis, cervicitis (89%).

In the group of erosion HSV *I, II* was defined with high frequency: 15% and 20% correspondingly.

HSV *I, II* and *Citomegalovirus (CMV)* were significantly associated with cases of heavy dysplasia and cancer *in situ*: 67% and 35% correspondingly.

Pappillomavirus (HPV) was detected in cohort of women affected by erosion and malignant cervical cancer. Many epidemiological studies indicated relationship between HPV infection and cervical neoplasia. Genital HPVs are divided into low-risk (HPVs 6, 11, 42, 43, 44) and high-risk (HPVs 16, 18, 31, 33, 35). HPV 16 is the most frequently detected in cervical intraepithelial neoplasia grade 3 (CIN3) and invasive carcinoma, while 31, 33 and 35 may be associated with less aggressive precursor lesions [1]. In our investigation we identified HPV 6-11 and HPV 16-18, 31-33. The frequency of HPV 6-11 types in malignant lesions of the cervix were 23%, HPV *16-18* - 4%, HPV 31-33 was not detected in investigated samples.

Taking into consideration the fact that HPV infection in investigated population was not widely distributed it was assumed, that HPV infection is not the main risk factor for cervical cancer development in Kazakhstan population

An interaction between HSV and cervical carcinogenesis was investigated by several groups. HSV was shown not to play direct role in cervical carcinogenesis [2]. We did not find HSV in invasive cancer, but revealed the presence of HSV in case of heavy dysplasia and cancer *in situ* (67%). According to our hypothesis the role of HSV agent is in "making the proper conditions" for HPV-oncotransformation in cervical epithelium, but this viral agent did not participate in this tumor inducing process directly. This potential interaction between HPV and HSV is supported with many lines of evidence. First, herpetic lesions facilitate HPV access to the basal layer. Second, the inflammatory response induced by herpes may suppress the T helper cell mediated immune response. Third, herpes infection does induce the production of nitric oxide resulting in cellular DNA damage together with direct actions by herpes viruses on host cellular DNA. Fourth, herpes virus infection accelerates replication of HPV and increases the integration of HPV DNA sequences. [3]. Concerning role of Herpes simplex virus of 5 type - CMV in cervical cancerogenesis we can find many evidences of indirect participation of CMV infection in cervical tumorogenesis [4]. CMV presence in heavy dysplasia and cancer *in situ* cases (35%) and its absence in invasive cancer cases in investigated population confirm this statement.

Distribution of urogenital infection in groups

Group	Infectious agent	Distribution, %
Control	*Gardnerella vaginalis*	5
	Mycoplasma hominis	7
	Citomegalovirus	2
Inflammation processes	Chlamydia trachomais	18
	Mycoplasma hominis	28
	Mycoplasma genitalium	22
	Gardnerella vaginalis	25
	Ureaplasma urealiticum	44
	Trihomonas vaginalis	16
Erosion	*Trihomonas vaginalis*	0,8
	Herpes simplex virus I	15
	Herpes simplex virus II	20
	Herpes virus 6	6
	Citomegalovirus	4
	Pappillomavirus 6-11	3
	Pappillomavirus 16-18	8
	Pappillomavirus 31-33	0,8
Heavy dysplasia and cancer *in situ* Invasive cancer	*Herpes simplex virus (I, II)*	67
	Citomegalovirus	35
	Pappillomavirus 6-11	23
	Pappillomavirus 16-18	4
	Pappillomavirus 31-33	0

Studies in Finland [5], California [6] and Mexico [7] found that *Chlamydia trachomatis* increases the risk of cervical cancer development. It was suggested that this infection agent might be a cofactor of HPV risk factor in the etiology of cervical cancer, possibly by modulating the host's immunity and/or maintenance of chronic inflammation status [8]. Some studies in Honduras, the US, Colombia and Spain failed to demonstrate any association between sexually transmitted infectioous agents, especially *C. trachomatis*, and cervical cancer [9]. Our data did not demonstrate any association between bacterial agents, including *C. trachomatis*, and cervical cancer development.

In conclusion, Kazakhstan women population study showed presence of different types of bacterial, viral and protozoa agents in cases of inflammation and erosion processes in cervical epithelium. In pap smears, which were diagnosed as heavy dysplasia, cancer in situ and invasive cancer, only viral agents (HPV and HSV) were detected. The absence of non viral infectious agents in these smears can be explained by lack of proper conditions for their growth.

LITERATURE

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Резюме

Жұмыстың мақсаты Қазақстан популяциясындағы әйелдердің жатыр мойыны патологияларының дамуындағы әртүрлі урогенитальды инфекциялардың ассоциацияларын зерттеу болып келеді. Зерттеу материалы ретінде 1000 әйелдерден жинап алынған қалыпты және патологиялық жатыр мойнының эпителиялары пайдаланылды. Урогенитальды инфекциялардың кездесу жиілігі дайын жиынтықтарды қолдана отырып, ПТР әдісімен пайдаланылды. Бактериялар мен вирустар бар агенттер, сонымен қатар жатыр мойнының түрлі жағдайдағы патологияда кездесетін қарапайымдылар анықталынды. Ауыр дисплазия, in situ жағдайында ісік, жатыр мойны ісігі ретінде диагнозы қойылған үлгілерде вирустық табиғаты бар агенттер ғана детекцияланған (HPV и HSV). Қазақстан популяциясындағы әйелдердің жатыр мойны эпителияларындағы онкологиялық патологиялардың дамуындағы адам папиллома вирусы басты фактор болып саналмайтынына болжам жасауға болады.

Резюме

Целью работы было изучение ассоциации различных урогенитальных инфекций с развитием патологий шейки матки у женщин, представляющих популяцию Казахстана. Материалом для исследования служили образцы нормального и патологического эпителия шейки матки, собранные у 1000 женщин. Встречаемость урогенитальных инфекций определяли методом ПЦР с использованием готового набора. Обнаружено присутствие агентов бактериальной и вирусной природы, а также простейших при различных фоновых патологиях шейки матки. В образцах, диагностированных как тяжелая дисплазия, cancer *in situ*, рак шейки матки детектированы только агенты вирусной природы (HPV и HSV). Следует предположить, что вирус папилломы человека не является главным фактором риска развития онкологических патологий эпителия шейки матки у женщин популяции Казахстана.

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