



Activity of Apoptosis Markers in HPV-Associated Cervical Pathologies

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Abstract

Difficulties in managing patients with cervical intraepithelial neoplasia (CIN) encountered in the work of practicing physicians are determined, firstly, by the lack of uniform treatment and diagnostic measures for cervical precancer and its prevention, and secondly, with insufficient understanding of the processes of human papillomavirus (HPV) -induced squamous intraepithelial lesions, mechanisms of action of anti-HPV drugs and, as a consequence, irrational ways of affecting the cervical tissue. In this regard, it seems relevant to develop and clinically test the use of etiopathogenetic effects on the initiating factors and cofactors of oncogenesis in order to create an optimal-radical effect that allows achieving complete regression of the pathological focus. This will reduce the frequency of neoplastic transformation of the cervix in women of the reproductive period, as well as improve the results of treatment and improve the quality of life of patients.

Keywords: Ectocervical Dysplasia; Caspase-1; Caspase-3; Caspase-9; Apoptosis-resistant Phenotype

Introduction

Despite numerous studies, diagnostic criteria and treatment options, the general pathology of gynecological morbidity includes pathologies of the cervix [1]. Currently, the most urgent problem in the field of obstetrics and gynecology is the combination of changes in the cervix with the activity of the human papillomavirus (HPV), with a frequent outcome in malignancy. Various types of virus are diagnosed in 97% of confirmed cervical cancers [4]. Relatively recently, the world health organization introduced screening programs aimed at the earliest possible diagnosis of pathological changes in the cervix against the background of HPV [1,2,5]. With the progression of the pathology of the cervical zone, HPV DNA is introduced into the cell genome [7,9,13], thereby provoking proliferation processes [8,10,21]. However, it is not

fully understood at what stage the virus influences the cellular genome and what causes the cell to change its natural processes, for example, apoptosis. It was found that during the formation of cervical cancer, there is a decrease in the activity of apoptotic mechanisms, where the leading role is given to caspases, the main enzymes [6,15,17-19]. Therefore, the formation of the correct tactics for managing patients based on the activity of apoptosis markers is the basis for effective preventive measures for cervical cancer.

Purpose of the study is to determine the degree of the apoptotic program violation by evaluating caspase-1, caspase-3 and caspase-9 expression in cervical mucus in HPV-positive patients of fertile age with cervical intraepithelial neoplasia I (CIN I).

Materials and Research Methods

For the study, 95 women were selected at the age from 18 to 45 years. The main study group included 55 patients with mild cervical intraepithelial neoplasia associated with human papillomavirus. The control group consisted of 40 healthy women.

When working with patients, all the requirements for medical research at universities in the Russian Federation were observed.

In the period from 2018 to 2019, a study was conducted of 95 women who applied for an outpatient clinic in Penza. In order to search for new approaches to the treatment of human papillomavirus infection, the factors of the cytokine response and markers of apoptosis in CIN I degree against the background of papillomavirus infection were assessed and their changes were analyzed in comparison with conventionally healthy women with normal findings of an oncocytopathological smear from the cervix without HPV. The criteria for inclusion in the groups with pathological conditions of the cervix were: the presence of high-risk HPV virus replication in the cervical canal, established by polymerase chain reaction (PCR); CIN I, histologically confirmed; absence in the last 6 months before the start of the study of therapy with drugs that could affect the results of the study; adequate contraception for women of childbearing age (using a barrier method of contraception); written informed voluntary consent to participate in the study; transformation zone 1 or 2 type; "Normocenosis" based on the results of the assessment of the lower part of the gynecological tract; patient compliance. The exclusion criteria were: age under 18 and over 45; pregnancy, lactation; severe somatic pathology; taking medications that could affect the studied parameters; the presence of decompensated diseases or acute conditions, including concomitant mental illness; the presence of other sexually transmitted infections; inability to follow the terms of the protocol.

In order to assess the cellular composition and detect the presence of atypical epithelial cells, a cytological examination of smears from the cervix of the examined women was carried out. The material for cytological examination was scraping from the cervical canal, transformation zone and from the surface of the cervix, obtained using a disposable cervical brush. The staining of cervical smears was performed according to the Pap test. The evaluation of the results of the cytological study was carried out in accordance

with the general provisions of the Bethesda Informative Classification System (The Bethesda System), developed in the USA in 1988.

All patients underwent colposcopy to assess the state of the cervix. If atypical changes were detected, a multifocal biopsy was performed to establish a histological diagnosis. All results were documented and recorded graphically.

The detection and differentiation of DNA of human papillomaviruses was carried out by PCR with hybridization fluorescence detection «AmpliSens® HPV VKR screen-titer-FL», according to the recommendations of the manufacturer FBSI «TsNIE» Rospotrebnadzor (Moscow). The reagent kit is intended for the detection and quantitative determination of HPV DNA of high carcinogenic risk (HRS) of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 types in clinical material. The method is based on the simultaneous amplification of HPV DNA regions and a DNA region of the β -globin gene, which was used as an endogenous internal control, with hybridization-fluorescent endpoint detection (FEP format). Scraping discharge of the cervical canal was used as a material for PCR diagnostics.

To determine the expression of the level of caspase 3 and caspase 9, we used an enzyme-linked immunosorbent assay with a set of reagents from Cloud. corp., the level of the studied caspases was assessed by the sandwich method for tissue homogenates.

Statistical processing of indicators was carried out using assessment methods and using the STATISTICA 9.0 program. Descriptive statistics were also used in the processing of the obtained data. When carrying out statistical indicators, the Fisher method was used - the method of angular transformation. The difference between the compared data was considered significant at $P > 0.05$.

Results and Discussion

According to the results of the research during the observation, the examined women were divided into groups. Among all examined patients, 55 women were verified to have HPV-positive cervical intraepithelial lesion of the 1st degree. They made up group I. The remaining 40 women with a normal cytological picture did not have HPV and, accordingly, had the NILM category according to oncocytopathology. The last group of patients constituted the control

group. Examination of this group made it possible to obtain the results of the physiological norm of the studied parameters. The age of the examined women varied from 19 to 45 years. So the average age in the 1st group of women with CIN I was 27.05 ± 0.51 , in the control group 30.41 ± 1.07 .

The first stage was a cytological examination of the patients. The overwhelming majority of women had normal cytological smears in both groups. In analyzing the data of cytological examination, 38 patients (69%) had normal results, 15% (8 patients) had low-grade squamous intraepithelial lesion (LSIL), and HSIL was found in 16% (9 patients). To determine the nature of the lesions of the cervical zone, an extended colposcopic examination was carried out. The main criterion for inclusion in the study was the presence of a type I or II transformation zone, which made it possible to fully assess the state of the cervix. All HPV positive patients with CIN I were diagnosed with an abnormal colposcopic picture. Mild lesions (grade I) on the cervix were diagnosed in 36 patients, which was 65%, and severe lesions (grade II) - in 19 patients (35%).

When analyzing the studied parameter, it was found that all patients with CIN I had combinations of several types of HPV and a clinically significant viral load (3-5 lg, > 5 lg). In 100% of the examined patients, HPV type 16 was diagnosed; HPV type 18 was detected in 95%; 31 types - 91%; Type 33 - in 67%; 35 type - 60%; 39 HPV type is identified in 40%; Type 52 - in 36% of patients and type 45 was diagnosed in 31%. Then the analysis of the correlation dependences was carried out.

To establish a final diagnosis, a multifocal cervical biopsy with a histological examination was performed. The results of this histological examination served as the basis for the formation of research group.

Thus, the analysis of colposcopic, cytological and histological results allows concluding that the histological method gives the largest information on the state of the cervical epithelium in HPV-positive patients with CIN I.

To determine the severity degree of the cervical changes and to evaluate the risk of developing atypical processes, an assessment of apoptotic process in the cervical mucus obtained during the cytological intake of the material was carried out. Determination of the level of caspase-1, caspase-3 and caspase-9 (Table 1) was another stage of the research.

Indicator	Caspase Level 1	Caspase Level 3	Caspase Level 9
Control group n = 40	$0,060 \pm 0,01$ ng/ml	$0,188 \pm 0,04$ ng/ml	$0,213 \pm 0,03$ ng/ml
group, n = 55	$0,062 \pm 0,03$ ng/ml	$2,772 \pm$ $0,03^*$ ng/ml	$2,311 \pm 0,05^*$ ng/ml

Table 1: The level of caspases in research groups.

Note: The assessment of the statistical significance of changes in the activity of caspase 3, caspase 9 and caspase 9 was carried out in accordance with the Wilcoxon-Mann-Whitney criteria. The symbol * marks the groups in which the caspase activity significantly differs from the "control" group (the differences are considered significant with $p < 0.05$).

Since the formation of dysplasia is the result of a violation of the natural balance between the processes of proliferation and cell death (apoptosis), the available data on the study of various mechanisms of the formation of resistance of tumor cells to apoptotic factors are ultimately aimed at suppressing the activity of these processes. In this regard, it is important to study the relationship between these indicators and parameters characteristic of dysplasia of the 1st severity [6,11,12,14].

The caspase 1 values in the study group I were equal to 0.062 ± 0.03 ng/ml, and did not differ statistically from the control group, that is, they were at the limit of the method's sensitivity. Since caspase 1 plays a leading role in the activation of the inflammatory response, the data obtained make it possible to conclude that patients with CIN I lack an inflammatory component in the formation of pathological changes in the cervix.

In clinical conditions, an assessment of violation degree of the apoptotic program in HPV-associated cervical pathology by determining caspase-3 and caspase-9 expression was carried out. Studying effector caspase-3 and caspase-9, changes were obtained characterizing the progression degree of HPV presence in cervical epithelial cells. Due to the lack of normal values for caspase under study, the results in the immunological control group were taken as normal indicators. In research groups, the activity of effector caspase-3 and caspase-9 was higher relative to the control level and increased as cervical pathology progressed against the background of the virus. These results confirm the formation of apoptosis resistant process in HPV-affected cells [9,11,12,21] due to activation of

caspace genes in changed areas of the cervical zone. It supplements the previous studies confirming the data on atypical progression with CIN III in cervical cancer, and an increase in caspase-3 level and caspase-9 level [13,15].

An increase in severity degree of a pathological process in the cervix was accompanied with determination of caspase-3 and caspase-9 expression (Table 1). This is explained by the fact that there are inactive monomeric precursors in the caspase cells, requiring cleavage of proenzyme and subsequent dimerization for activation. The implementation of these reactions is possible with successive mutual activation of caspases. The starting moment for such transformations is DNA damage, which in turn launches a caspase cascade. It can follow either external or internal pathway, but no matter what pathway the cascade is launched, caspase-3 and caspase-9 is its effector caspases. The mechanisms for HPV-associated cervical pathologies formation, such as CIN I, can be considered as expression of effector caspase-3 and caspase-9 levels. The studied cysteine protease is able to increase with introduction of HPV DNA into the host cell. Determination results of caspase-3 and caspase-9 have shown different values in the expression level of apoptosis marker in the cervical epithelium as HPV-associated pathology progresses.

Determining the level of caspase-3 and caspase-9 in case of CIN I proves the validity of an in-depth dispensary observation and eventual active treatment of these patients. The obtained data on change of the apoptotic program and, as a consequence, local immunity are a pathogenetic justification for complex therapy using antiviral drugs with an immunomodulatory effect.

Conclusion

A key role in the process of pyroptosis is played by cytokines, which, when released, induce an inflammatory process. One of the final stages to implement inflammatory reactions is caspase-1. The indicators were not statistically different when determining its level in control and research groups, which points out the absence of inflammatory cervical process in HPV-positive patients with CIN I.

For a long time, reparative processes have been limited exclusively by epithelium of the cervical zone under the influence of

human papillomavirus. Introducing HPV DNA into the host cell, a cascade of apoptotic reactions is launched, where the final stage is activation of effector caspase-3 and caspase-9. The determination results of caspase-3 and caspase-9 have shown different values in the expression level of apoptosis marker in the cervical epithelium as HPV-associated pathology progresses. Therefore, an assessment of cervical zone by means of determining the level of apoptotic caspase-3 in HPV-positive patients are prognostic and diagnostic criteria for assessing the severity of the identified pathology.

Conflict of Interest

There is no conflict of interest.

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