DOI 10.26724/2079-8334-2021-2-76-89-93
UDC 616.6-006-07

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THE ROLE OF IMMUNOGOHISTOCHEMICAL STUDY METHODS IN THE DIAGNOSIS OF PROSTATIC INTRAEPITELIAL NEOPLASIA

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The article deals with the problem of diagnosing prostatic intraepithelial neoplasia as a precancerous process. Data on the expression of proliferation markers (Ki-67) and invasion (p63) in patients with prostatic intraepithelial neoplasia of high and low degrees are analyzed. The total of 93 patients with prostatic intraepithelial neoplasia who were studied for Ki-67 and p63 expression were examined. High-grade prostatic intraepithelial neoplasia has been shown to have a greater potential for proliferation and invasive growth than low-grade prostatic intraepithelial neoplasia. In patients with high-grade prostatic intraepithelial neoplasia, more intensive expression of Ki-67 was observed by 24 % more frequently, and by 11 % pronounced expression of Ki-67 than in patients with low-grade prostatic intraepithelial neoplasia. P63 expression was higher in cases of low-grade prostatic intraepithelial neoplasia. In patients with low-grade prostatic intraepithelial neoplasia, intense p63 expression was by 25 % more common and pronounced p63 expression was by 14 % more common. The data suggest the need for in-depth examination of patients with high-grade prostatic intraepithelial neoplasia to prevent malignancy.

Key words: high and low degree prostatic intraepithelial neoplasia, immunohistochemical markers.

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РОЛЬ ІМУНОГІСТОХІМІЧНИХ МЕТОДІВ ДОСЛІДЖЕННЯ У ДІАГНОСТИЦІ ПРОСТАТИЧНОЇ ІНТРАЕПІТЕЛІАЛЬНОЇ НЕОПЛАЗІЇ

Стаття стосується проблеми діагностики простатичної інтраепітеліальної неоплазії як передракового процесу. Аналізуються дані експресії маркерів проліферації (Ki-67) та інвазії (p63) у пацієнтів з простатичною інтраепітеліальною неоплазією високого та низького ступенів. Обстежено 93 пацієнта з простатичною інтраепітеліальною неоплазією, яким було проведено вивчення експресії Ki-67 та p63. Встановлено, що простатична інтраепітеліальна неоплазія високого ступеня має більший потенціал проліферації та інвазивного росту, ніж простатична інтраепітеліальна неоплазія низького ступеня. У пацієнтів з простатичною інтраепітеліальною неоплазією високого ступеня на 24 % частіше зустрічалася інтенсивна експресія Ki-67 та на 11 % частіше спостерігалася виражена експресія Ki-67, ніж у хворих з простатичною інтраепітеліальною неоплазією низького ступеня. Експресія p63 була більшою у випадках простатичної інтраепітеліальної неоплазії низького ступеня. У пацієнтів з простатичною інтраепітеліальною неоплазією низького ступеня на 25 % частіше зустрічалася інтенсивна експресія p63 та на 14 % частіше спостерігалася виражена експресія p63. Дані свідчать про необхідність поглибленого обстеження пацієнтів з простатичною інтраепітеліальною неоплазією високого ступеня, для попередження малігнізації.

Ключові слова: простатична інтраепітеліальна неоплазія високого та низького ступеня, імуногістохімічні маркери.

The work is a fragment of the research project "Improvement of specialized and highly specialized medical care of surgical profile on the principles of "rapid surgery" for certain diseases of the thyroid and parathyroid glands, nasopharynx, internal and reproductive organs, abdominal wall, blood vessels and joints, in particular using atomic force microscopy and prelamination method for implant treatment", state registration No. 0119U001046.

Prostate cancer (PC) is a disease that is a pressing medical and social problem worldwide. According to the WHO, there are about 1,100,000 new cases of PC and about 300,000 deaths from this disease in the world every year [7]. The standardized incidence of men with PC in 2015 in Ukraine was 26.2 per 100 thousand population, and mortality made 11.4 per 100 thousand population. Over the past 15 years, there has been an increase in morbidity by 42 % and mortality– by 25 % [2].

One of the ways to improve the early diagnosis and treatment of PC is timely detection of precancerous conditions, their comprehensive study and development of algorithms for diagnosis, treatment and prognosis in patPINts with precancerous diseases. In recent years, researchers have paid considerable attention to determining the features of the prostate precancerous conditions, namely prostatic intraepithelial neoplasia (PIN). PIN is defined as a preinvasive pathological process that occurs as a result of intraglandular cell proliferation of the epithelium. At present, prostatic intraepithelial neoplasia is divided into low-grade PIN (PINLG) and high-grade PIN (PINHG) [12].

According to various authors, the frequency of PINs in the screening population ranges from 0.7 % to 20 %. At the same time, patPINts with suspected PC are more likely to detect PINs and range from 4.4 % to 25 %, and after TURP - from 2.8 % to 33 % [4, 5, 11].

Standard histological examination is not always sufficPINt for differential diagnosis and determination of the pathological process features in the prostate [10]. Immunohistochemical methods, in contrast to standard microscopy, provide specific imaging in various cell tissues , growth factors and their receptors, enzymes, immunoglobulins, cell components and even individual genes [6, 7].

An important task is to single out a subgroup of high risk of malignancy among the general mass of patPINts with PINs. The clinical behavior of precancerous processes, their ability to invade and metastasize depend on the molecular biological markers' balance, growth factors and inhibitory factors. [8, 9, 13]. In recent years, both the prognostic value of respective markers and the possibility of their use as targets in targeted therapy have been studied. At the same time, insufficPINt data on the molecular markers' clinical significance in PIN patients with precancerous prostatic diseases is noteworthy. The p63 marker is expressed in the prostatic tissue exclusively by the basal layer of the epithelium. In PC, the expression of p63 is significantly reduced, which is detected by immunohistochemical study [15]. To assess the biological activity of tissues, Ki-67 is used (proliferative activity), which belongs to the regulatory proteins, its occurrence coincides with the entry of the cell into mitosis, permitting to use it as a universal marker of proliferation to assess the growth of malignant tumors.

The Ki-67 index is an independent indicator to prognose relapse and survival of PIN patients with PC; there is a direct correlation between the number of tumor cells expressing Ki-67 and the stage of PC [1, 3, 14].

The purpose of the study was to determine the expression of immunohistochemical markers of proliferation and invasion in patPINts with low and high grade prostatic intraepithelial neoplasia.

Materials and methods. The work is based on the analysis of examination and treatment results in 93 PIN patients with stage I-III benign prostatic hyperplasia (BPH) with the presence of PINLG and PINHG, who were treated at the center of minimally invasive surgery SRI "SPC PCM", SMA and the urology department of Zaporizhzhya Regional Clinical Hospital from February 2009 to May 2014. The age of PIN patients ranged from 55 to 72 years (M=66±1.48). The diagnosis of PIN was established by histopathological examination of microslides obtained by PG biopsy or TURP.

By means of immunohistochemical study methods, the molecular and biological properties of PIN in terms of its ability to malignant transformation were studied. The expression of the Ki-67 proliferative activity marker and the p63 marker of invasive growth by immunohistochemical method was studied. In the assessment of immunohistochemical staining, a semi-quantitative method was used to study the Ki-67 and p63 markers expression in tissue with 4 categories, assigning the appropriate reaction values from "+" to "++++" or from 1 to 4 points. A score of "+" was assigned to an unexpressed reaction, "++" indicated a weak color, "+++" was a moderate color, and "++++"indicated an intense color.

A comprehensive immunohistochemical study examining the expression of proliferation markers (Ki-67) and invasive growth (p63) was performed in 93 patients. Among those examined were 50 patients with PINHG and 43 people with PINLG.

The obtained results were subjected to statistical processing using the methods of variation statistics with "Penthium 4" computer using the software package "Statistica" (version 6.0, StatSoft Inc, USA). For comparative analysis of the samples, the probability of difference was confirmed using the nonparametric Mann-Whitney test for independent populations. The difference $p<0.05$ was considered statistically significant.

Results of the study and their discussion. The expression of Ki-67 and p63 analysis was performed separately in patients with low- and high-grade PINs to identify differences in immunohistochemical parameters and their correlation with clinical features.

During the analysis of Ki-67 proliferation marker's expression by means of a semi-quantitative method, it was found that in patients with PINHG in 24 (48 %) cases intensive Ki-67 expression was detected at the level of "++++", in 15 cases (30 %) Ki-67 was "+++" (moderate), in 6 cases (12 %) the expression of Ki-67 was "+" (unexpressed) and in 5 cases (10 %) the expression of Ki-67 was detected at the level of "++" (weak expression). Thus, in patients with PINHG established the following patterns regarding the level of expression of Ki-67: most often determined by the intense expression, which is characteristic of high level cell proliferation, including prostate adenocarcinoma. Moderate expression of Ki-67 was by 18 % less common and was also characteristic of malignant neoplasms. Unexpressed and weak expression in patients with PINHG was observed with the lowest frequency.

One of the study objectives was to establish the value and informativeness of p63 invasion marker's expression in the PIN diagnosis in terms of the ability of the precancerous process to invade. During the analysis of the p63 invasion marker's expression, it was found that in patients with PINHG in 22 (44 %) cases the weak expression of p63 was found at the level of "++", in 14 cases (28 %) the expression of p63

was moderate "+++". In 9 cases (18 %) p63 expression was unexpressed "+" and in 5 (10 %) cases intensive p63 expression was detected at the level of "++++". The results of immunohistochemical study in patients with PINHD are presented in table 1.

Таблиця 1.

Expression of Ki-67 and p63 markers in patients with PINHD

Marker	+	++	+++	++++
Ki-67	6 (12 %)	5 (10 %)	15 (30 %)	24 (48 %)
P63	9 (18 %)	22 (44 %)	14 (28 %)	5 (10 %)

Thus, in patients with PINHG were established the following patterns concerning the level of p-63 expression: most frequently were determined weak expression, and the least frequently – intense expression, which is characteristic of tumors that have the ability to invasive growth.

In patients with PINLG the following data of immunohistochemical research are established. Ki-67 expression was characterized as follows: the most common weak expression of Ki-67 "++"– 19 (44 %) cases, unexpressed expression of Ki-67 "+"– 10 (23 %) observations, moderate expression of Ki-67 "+++"– 8 (19 %) cases, intensive expression of Ki-67 "++++ " – 6 (14 %) cases. Thus, in patients with PINLG, weak and unexpressed expression of the proliferation marker was more common than moderate and intense, which is characteristic of benign tissue.

Regarding the expression of p63 in patients with PINLG, the following data were found: moderate p63 expression "+++" was most frequently observed – 21 (42 %) cases, intensive p63 expression "++++"– 20 (40 %) cases. Less frequently, p63 unexpressed expression "+" was found – 5 (10 %) cases, p63 weak expression "++"– 4 (8 %) cases. Therefore, the results of immunohistochemical studies in patients with PINLG are presented in table 2.

Таблиця 2.

Expression of markers Ki-67 and p63 in patients with PINLG

Marker	+	++	+++	++++
Ki-67	10 (23 %)	19 (44 %)	8 (19 %)	6 (14 %)
P63	5 (10 %)	4 (8 %)	21 (42 %)	20 (40 %)

Thus, in patients with PINLG, moderate and intense expression of p63 invasive growth marker (42 % and 40 %, respectively) was most frequently determined. This pattern is inherent of benign tissue.

When comparing the level of Ki-67 expression in patients with PINHG and PINLG, significant differences were found. Thus, weak expression of Ki-67 "++" occurred in patients with PINNS by 34 % more frequently than in patients with PINHG, unexpressed expression of Ki-67 "+" was observed in patients with PINLG by 11 % more frequently than in patients with PINHG ($p<0.05$). At the same time, patients with PINHG were by 24 % more likely to express Ki-67 and by 11 % more likely to express Ki-67 ($p<0.05$) (fig. 1).

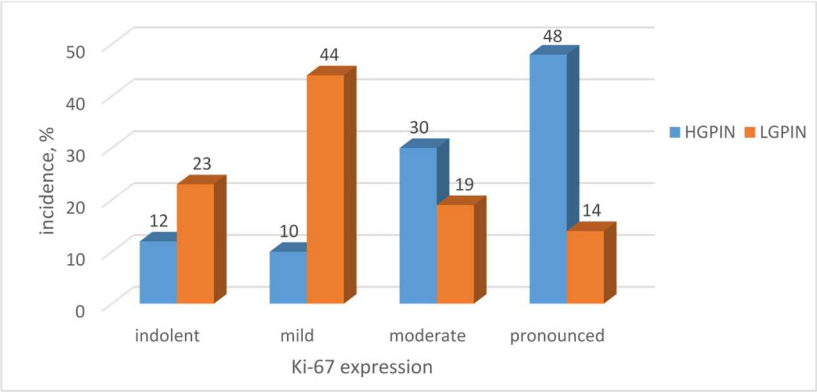

Fig. 1. The level of Ki-67 expression depending on the PIN degree

In a comparative analysis of the p63 expression level in patients with PINHG and PINLG, it was found that the p63 "++" expression occurred in patients with PINHG by 28 % more frequently than in patients with PINLG, p63 "+" expression was observed in patients with PINLG by 11 % more frequently than in patients with PINHG ($p<0.05$). At the same time, in patients with PINLG intensive expression of p63 was by 25 % more common and pronounced expression of p63 was observed by 14% more frequently ($p<0.05$) (fig. 2).

Thus, the results of the study on the immunohistochemical proliferation (Ki-67) and invasive growth (p63) markers' expression established statistically significant differences between patients with high and low PIN. Patients with PINHG were characterized by significantly higher values of Ki-67 markers and significantly lower values of p63 than in patients with PINLG. The obtained data confirm the common morphological characteristics of PINHG and PC, greater susceptibility of PINLG to malignant transformation in comparison with PINLG, which is a prerequisite for a higher probability of PC detection in patients with PINLG during the observation period.

Despite the fact that PINs are considered by many researchers to be a precancerous condition of the prostate, the prevailing opinion among scientists and practical urologists is that it is inexpedient to use active tactics of monitoring and treatment of PIN patients. At the same time, there are data on malignant progression and detection of PC, in particular with the presence of distant metastases, in patients with PIN during dynamic monitoring. It is obvious that patients with IDUs are a heterogeneous group with different risk of malignancy.

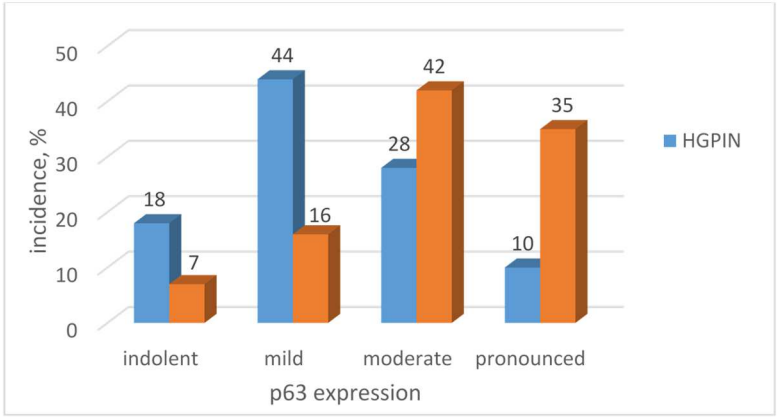

Fig. 2. The p63 expression level depending on the PIN grade.

The urgent task is to stratify patients with PINs for the purpose of their differentiated examination, observation or treatment. The role of immunohistochemical study methods based on the studying markers of growth, progression, invasion and their receptors is to establish the potential for malignant transformation in each individual patient with PIN and individualization of tactics for in-depth examination and treatment. The identified differences in the expression intensity of immunohistochemical markers reflect the diversity of PINs' biological properties, which determine the probability of malignant transformation.

According to Magi-Galuzzi C. et al., immunohistochemical study is decisive in the differential diagnosis of pancreatic diseases and permits to identify their biological portrait [10]. The expression level of proliferation and invasion markers in patients with PINHG has common characteristics with prostate adenocarcinoma, which is a sign of high ability of PINHG foci to malignancy. Adisa J et al. found that benign PG diseases have common immunohistochemical characteristics with PC, but the expression level of Ki-67 is a significant factor in malignancy [1].

According to Kovylina et al., Ki-67 expression is a prognostic factor in PC. Intense expression of Ki-67 has a direct correlation with the incidence of locally advanced forms of adenocarcinoma, regional and distant metastases [14]. Verma R. et al. found greater expression of Ki-67 in malignant tumors of the prostate than in benign conditions such as benign prostatic hyperplasia. In the study of the PC relapse incidence, after radical treatment and expression of Ki-67, a correlation was found between the level of Ki-67 expression and the recurrence rate of PC [13].

Patients with PINHG, high Ki-67 expression and low p63 expression are at risk and require in-depth examination and monitoring. In addition, the established patterns affect the choice of observation and treatment tactics in patients with PIN, namely they indicate the need not only for in-depth examination of patients with PIN, but also the assignment of therapy to prevent malignant transformation of precancerous prostate pathology in such patients.

Conclusions

1. Intensive expression of Ki-67 was by 24 % more common in patients with PINHG and pronounced Ki-67 expression was by 11 % more frequent than in patients with PINLG.

2. In patients with PINLG intensive expression of p63 was by 25 % more common and expressed expression of p63 was observed by 14 % morefrequently.

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Стаття надійшла 28.04.2020 р.

DOI 10.26724/2079-8334-2021-2-76-93-98
UDC 618.396-085.356:577.164.17-084

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FOLATE CYCLE DRUGS IN THE COMPLEX PREVENTIVE THERAPY FOR THE MISCARRIAGE

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120 women of reproductive age were examined. Alleles of folate-related enzyme genes, the content of folic acid, cyanocobalamin, vitamins B_1, B_6 in the blood were determined. Prevention of miscarriage was based on preconception training of men and women (spouses). The pre-conceptual examination plan included a thorough study of the anamnesis, including family history; laboratory determination of folic acid, vitamins B_{12}, B_1, B_6 in the blood of future parents (both woman and man). Decreased vitamin content or their level at the lower limit of normal was an indication for determining the folate-related enzyme genes (*MTHFR: 1298A/C; 677C/T, MTR: 2756A/G*, *MTRR: 66A/G*). Given the high (78.1 %) incidence of carriers of "functionally attenuated" (polymorphic) genes of folate cycle enzymes, both expectant parents were prescribed modern complexes of vitamins, microelements and amino acids containing Metafolin at the stage of preconception preparation 3-4 months before fertilization and during pregnancy according to the trimesters of gestation. Clinical and laboratory approach to the correction of vitamins, macro-, microelements, amino acids based on laboratory assessment of hereditary impairment of specific enzymes and by prescribing personalized therapy had a positive result in 57 (95.0 %) patients, which confirms its effectiveness.

Key words: folic acid, vitamins B_{12}, B_1, B_6, alleles of folate-related enzyme genes, prevention of miscarriage.

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ПРОФІЛАКТИКА НЕВИНОШУВАННЯ ВАГІТНОСТІ ШЛЯХОМ ЗАСТОСУВАННЯ ФОЛАТІВ У КОМПЛЕКСНІЙ ТЕРАПІЇ

Обстежено 120 жінок репродуктивного віку. Визначено алелі генів ферментів фолатного циклу, вміст фолієвої кислоти, ціанокобаламіну, вітамінів B_1, B_6 в крові. Профілактика невиношування вагітності базувалась на преконцепційній підготовці чоловіка та жінки (подружжя). До плану обстеження в межах преконцепційної підготовки включалось ретельне вивчення анамнезу, в тому числі сімейного; лабораторне визначення вмісту фолієвої кислоти, вітамінів B_{12}, B_1, B_6 у крові майбутніх батьків (і жінки, і чоловіка). Знижений вміст вітамінів або їхній рівень на нижній межі норми був показанням до визначення генів ферментів фолатного циклу (*MTHFR: 1298A/C; 677C/T, MTR: 2756A/G, MTRR: 66A/G*). Враховуючи високу (78,1 %) частоту зустрічаємості носіїв «функціонально ослаблених» (поліморфних) генів ферментів фолатного циклу, обом майбутнім батькам на етапі преконцепційної підготовки призначались сучасні вітамінно-мікроелементні-амінокислотні комплекси, що містять Метафолін, за 3–4 місяців до запліднення та під час вагітності за триместрами гестації. Клініко-лабораторний підхід до корекції вмісту вітамінів, макро-, мікроелементів, амінокислот на підставі лабораторної оцінки спадкового порушення активності специфічних ферментів та шляхом призначення персонофікованої терапії мав позитивний результат у 57 (95,0 %) обстежуваних, що підтверджує його ефективність.

Ключові слова: фолієва кислота, вітаміни B_{12}, B_1, B_6, алелі генів ферментів фолатного циклу, профілактика невиношування.

The study is a fragment of the research project "The role of chronic infection of the uterus and lower genital tract in the formation of obstetric and gynecological pathology", state registration No. 0117U005276.

The prevention of miscarriages (PM) is one of the most important tasks of medicine today. The urgency of the problem lies in the high frequency of this pathology and the negative consequences for the woman's body. Premature termination of pregnancy at different times can contribute to the occurrence of gynecological (cervical incompetence, infectious processes of the uterus, cervix and uterine appendages, vagina, etc.) and somatic (anemia, neuroses, vascular diseases, etc.) pathologies. In addition, reproductive loss is a severe psychological trauma for a woman, her husband, and family members. Primary premature termination of pregnancy, habitual miscarriage (2 or more times) by pathogenetic mechanism is a