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IMPROVEMENT OF METHODS FOR EARLY DIAGNOSIS AND PREDICTION OF GESTATIONAL TROPHOBLASTIC DISEASE

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✓ ***Resume***

This study evaluated the expression of the CLIC-1 protein by immunohistochemical method in gestational trophoblastic disease, as well as its diagnostic value in predicting the malignant process. The study included 71 patients with gestational trophoblastic disease. There were higher levels of CLIC-1 immunoreactivity in trophoblastic cells in patients with gestational trophoblastic neoplasia compared with patients with hydatidiform mole. Thus, CLIC-1 can serve as a prognostic marker that allows detecting malignant transformation at an early stage.

Key words: gestational trophoblastic disease, hydatidiform mole, choriocarcinoma, immunohistochemistry, CLIC-1.

СОВЕРШЕНСТВОВАНИЕ МЕТОДОВ РАННЕЙ ДИАГНОСТИКИ И ПРОГНОЗИРОВАНИЯ ГЕСТАЦИОННОЙ ТРОФОБЛАСТИЧЕСКОЙ БОЛЕЗНИ

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✓ ***Резюме***

В исследовании оценивалась экспрессия белка CLIC-1 иммуногистохимическим методом при гестационной трофобластической болезни, а также его диагностическая ценность в прогнозировании злокачественного процесса. В исследование было включено 71 пациентов с диагнозом гестационная трофобластическая болезнь. Наблюдались более высокие уровни иммунореактивности CLIC1 в трофобластических клетках у пациенток с гестационной трофобластической неоплазией по сравнению с пациентками с пузырным заносом. Таким образом, CLIC1 может служить прогностическим маркером, позволяющим на ранней стадии выявлять злокачественную трансформацию.

Ключевые слова: гестационная трофобластическая болезнь, пузырный занос, хориокарцинома, иммуногистохимия, CLIC-1.

GESTATION TROPHOBLASTIK KASALLIKNI ERTA TASHHISLASH VA PROGNOZLASH USULLARINI TAKOMILLASHTIRISH

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✓ ***Rezyume***

Ushbu tadqiqotda CLIC-1 proteinining ekspressiyasi gestatsion trofoblastik kasalligida immunogistokimyoviy usul bilan baholandi va malignizatsiya jarayonini prognozlashda uning diagnostik ahamiyati o'rganildi. Tadqiqot gestatsion trofoblastik kasalligi bilan kasallangan 71 ta bemorni o'z ichiga olgan. Yelbo'g'oz bo'lgan bemorlarga nisbatan gestatsion trofoblastik neoplazisi bo'lgan bemorlarda trofoblastik hujayralardagi CLIC-1 ning yuqori darajadagi immunoreaktivligi kuzatildi. Shunday qilib, CLIC-1 erta bosqichda yomon sifatli o'zgarishlarni aniqlash imkonini beradi va prognostik marker bo'lib xizmat qilishi mumkin.

Kalit so'zlar: gestatsion trofoblastik kasalligi, yelbo'g'oz, horiokarsinoma, immunogistokimyo, CLIC-1.

Relevance

Gestational trophoblastic disease (GTD) is a term that unites a group of pathological conditions associated with pregnancy that develop as a result of abnormal trophoblast cell proliferation after fertilization [1,6]. Includes benign forms: hydatidiform mole (HM, complete or partial) and malignant forms: invasive mole, placental site trophoblastic tumor, epithelioid trophoblastic tumor, and choriocarcinoma. [2,3]. The European Organisation for Treatment of Trophoblastic Diseases (EOTTD) and the International Society of the Study of Trophoblastic Diseases (ISSTD) proposed to classify complete and partial HM as precancerous conditions and register as stage 0 of malignant trophoblastic tumors [2,3,8].

There is a geographic diversity in the distribution of GTD. In the structure of oncogynecological pathology, the frequency of malignant neoplasms of the placenta ranges from 0.1 to 3.6%. The incidence rate varies widely - from 0.01% ooo in Africa, America, Europe, England, Canada and others to 2.2% ooo in Vietnam. In East Asian countries, trophoblastic tumors are found 30-40 times more often than in Europe, and CC is observed, respectively, in 42.0-70.0% of patients. According to the WHO, the number of annually registered cases of the disease is underestimated [1, 6, 10].

Human chorionic gonadotropin β (β-hCG) is currently used as a biomarker for GTD. Monitoring of β-hCG is the main method for assessing the process of transformation from benign to malignant forms of the disease [2,3]. However, there is no diagnostic method to predict this transformation.

Given that 20% of complete and 5% of partial HMs progress to gestational trophoblastic neoplasia (GTN), there is always great concern about the prediction and early diagnosis of this transition. Many studies have been reported in the field of HM and factors predicting its progression to GTN, including, for example, assessment of the histopathological features of molar pregnancies, the level of the free β-hCG subunit, the assessment of the Ki67 and CA-125 marker, and the level of telomerase in HMs tissue samples. Various studies have studied the effect of PCNA, MMP, nm23, P16, HFC-1, DAPK, E-Cadberic, BCL-2, Rb, and mdm2 genes on evolution and transition to GTN [2-8].

Recently, the CLIC1 protein has been widely used among researchers. The functions of the CLIC1 protein range from ion homeostasis to regulation of cell volume, transepithelial transport, and regulation of electrical excitability [10]. Overexpression of CLIC1 has been found to be highly correlated with lymph node metastasis, lymphatic and perineural invasion, and poor survival. It has been suggested that CLIC1 overexpression modulates cell division and/or anti-apoptosis signaling leading to cell transformation [9].

A 2018 study in Poland used clinical proteomics [12]. Clinical proteomics is the identification and quantification of all individual proteins that are contained in a biological sample (serum, cerebrospinal fluid, urine, tissue) and monitoring changes in their concentrations. This study included the identification of 17 altered expression proteins, 11 of which (including septin 1, choriomammotropin, cytokeratin 8, and peroxiredoxin-2) were potential biomarkers of malignant transformation. In 2011, another study was carried out aimed at identifying prognostic biomarkers that indicate malignant transformation of the HM. The authors compared the protein profiles of HM with the profiles of malignant-transformed HM. 18 altered proteins were found in the malignant-transformed group. Among them, chloride intracellular channel protein 1 (CLIC1) was selected by the authors of the 2011 study for further study, the expression levels of CLIC1 in the choriocarcinoma tissue were higher than in the tissue of complete HM. The study showed that CLIC1 is a potential new prognostic biomarker that may indicate a high risk of malignant transformation of the HM [13]. Based on this, we can

conclude that proteomics is a promising method for detecting protein profiles and increasing the sensitivity and specificity of currently used biomarkers [12, 13,14,15].

At present, the epidemiology of GTD in Uzbekistan has been insufficiently studied, statistics only take into account its malignant forms, and there are no single centers for monitoring this disease. Absence of methodological principles for monitoring women after evacuation of the HM often leads to late detection of the disease, inadequate chemotherapy carried out in clinics that have no experience in treatment, with the subsequent development of resistant tumors and a worsening prognosis. To improve the quality of medical care for this category of patients in 2011, the European Society for the Treatment of Trophoblastic Disease (EOTTD) initiated the creation of a unified European "Protocol for the diagnosis and treatment of gestational trophoblastic disease (hydatiform mole and malignant trophoblastic tumors)" [10]. The paper analyzes the current state of the problem.

The relevance of the problem of GTD is quite high, since women, predominantly of young reproductive age, sometimes under 20 years old, are susceptible to this disease. In studies conducted in 2018, clinically significant increases in the level of anxiety, depression, sexual dysfunction and stress were found in women who underwent GTD [16]. The issues of prevention and successful treatment are closely related to the medical and social problems of maternity protection, intra-family relationships, and medical rehabilitation.

The aim of this study was to identify a prognostic biomarker indicating a possible malignant transformation of a hydatidiform mole.

Materials and methods

For this study, 71 patients with GTD were selected and divided into 3 groups: 27 samples of hydatidiform mole, 23 - invasive mole, 21- with choriocarcinoma, 25 women with non-developing pregnancy were selected as controls. The trophoblastic disease tissue samples used in this study were obtained from Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (Uzbekistan) and its affiliates. The tissues of the control group were obtained in the gynecological department of the Andijan Regional Perinatal Center. All diagnoses were histologically confirmed. Tissue samples were obtained by primary curettage of the uterine cavity or by trephine biopsy, washed with saline, and then immediately placed in formalin. The age of patients in our studies ranged from 16 to 55 years, the average age was 30.3± 2 years, with hydatidiform mole - 29.1±3, with invasive hydatidiform mole and choriocarcinoma - 32.3±4 years. These results indicate that malignant forms occur at a young reproductive age and in most cases lead to loss of fertility due to hysterectomy.

To achieve this goal, the method of immunohistochemistry was used. Formalin-fixed tissues were embedded in paraffin, separated by 5 μm, and mounted on silane-coated glass slides. Sections were deparaffinized and rehydrated through descending alcohol grades to distilled water, followed by endogenous peroxidase blocking with 3% hydroperoxidase in phosphate buffered saline; the sections were then exposed to microwave antigen. After that, they were washed in phosphate-buffered saline and blocked with rabbit serum (DAKO, Denmark) for 2 hours. They were then incubated overnight at 4°C with the CLIC1 polyclonal antibody (1:200 dilution, Elabscience Biotechnology Co., China).

After 3 washes in phosphate-buffered saline, sections were incubated with secondary peroxidase-conjugated antibody, (1:1000) for 1 hour at room temperature. Immunoreactivity was detected with diaminobenzadine (DAKO, Denmark) to increase sensitivity and form a brown insoluble precipitate in immunopositive areas. The sections were stained with hematoxylin and placed on a coverslip. Negative controls were incubated with a solution devoid of any primary antibodies. Tumor tissues of the corpus uteri were selected as positive controls according to the CLIC1 antibody protocol. The sections were stained with hematoxylin and placed on a coverslip. Negative controls were incubated with a solution devoid of any primary antibodies. Tumor tissues of the corpus uteri were selected as positive controls according to the CLIC1 antibody protocol. Sections were examined under a powerful (x400) light microscope. The 4 fields per section were randomly selected and the images were taken with a digital camera.

Sections were scored according to the percentage of CLIC1-stained trophoblast cells: 0 - for no positive cells, 1- for positive cells from 1% to 20%, 2- from 21% to 50%, 3 - from 51% to 80%, and 4 - from 81% to 100% of positive cells.

Result and discussion

According to the statistical data for the period 2011-2019, 161 cases of GTD were registered. Of these, 102 (63%) were HM, 9 (5,5%) were placental site trophoblastic tumors, 23 (14,2%) were invasive HM, and 27 (17%) were choriocarcinomas. Among the cases of HM, 70 (68.6%) were complete HM, 32 (31.3%) were partial HM.

The largest number of patients were from the Tashkent city (n = 28, 18.6%), the least number of patients were from Namangan region (n=3, 2%), probably this is due to the problem of diagnosis and insufficient histological examination of the abortion material.

There were 91 women living in rural areas (60, 6%), which confirms the authors' opinion about the correlation between trophoblastic disease and nutritional deficiency of mothers during the conception of their daughters and the development of HM during pregnancy of these daughters in the future. This is mainly a deficiency of vitamin A and / or B9 (folate) during the first week of development of the female fetus, which can disrupt the normal differentiation of their oocytes [17].

Most women (45%) had a history of spontaneous abortions and non-developing pregnancies. Comorbidities: iron deficiency anemia occurred in 90% of cases, urinary tract infection - 65% of women, colpitis and other inflammatory diseases of the genital organs - 75%.

A common clinical symptom in women with complete HM was uterine bleeding, signs of early pregnancy toxemia. There was an excessive increase in the size of the uterus relative to the gestational age. In 50% of women, ultrasound examination revealed theca lutein cysts larger than 6 cm, with bilateral localization. In most cases, beta-hCG levels were between 50,000 - 100,000 mIU / ml.

According to the FIGO classification (2000), the distribution of choriocarcinoma by stage was as follows: stage I – 9 (33.3%) patients, stage II – 6 (22.2%), stage III – 7 (26%), stage IV – 5 (18.5%). The outcome of previous pregnancy was HM in 16 (59.2 %) patients, abortion – in 9 (33.3 %), and childbirth – in 2 (7.4 %). The interval from the end of the last pregnancy to the manifestation of the disease varied from 1 month to 5 years.

To determine the early markers of malignancy of hydatidiform mole, we carried out an immunohistochemical study of tissues of various forms of GTD.

Table 1 shows the comparative results of immunohistochemical staining of the studied groups depending on the severity of expression of the click 1 protein.

Table 1. Comparison of CLIC1 expression in the studying groups

Groups	**0 (no staining)**	**1+(1 – 20%)**	**2+(21– 50%)**	**3+(51– 80%)**	**4+(>50%)**	**N**
Hydatidiform mole	13 (48,1%)	6 (22,2%)	2(7,4%)	4 (14,8%)	2(7,4%)	27
Invasive hydatidiform mole	0	1(4,3%)	3 (13%)	8 (34,0%)	11 (47,8%)	23
Choriocarcinoma	0	0	1 (4,7%)	2 (9,5%)	18 (85,7%)	21
Control group	18 (72%)	7 (28%)	0	0	0	25

Immunohistochemical study of the CLIC1 antigen in patients of group 1 diagnosed with hydatidiform mole showed the following results: of 27 patients, 13 (48.1%) had no expression, 22% of women had a weakly positive reaction, 7% had a moderately positive reaction and 6 samples (22.2%) showed strong cell staining (pic. 1).

Based on the results of an immunohistochemical study of hydatidiform mole, we concluded that 2 patients (7,4%), have a very high risk of malignant transformation.

In group 2, in 23 patients with a histological diagnosis of invasive mole, the results of the immunohistochemical study showed that the absence of reactivity was not observed in any case, 4% of women had a weakly positive reaction, 13% had a moderately positive reaction, and 34% had a positive reaction and in 11 cases (47.8%) strong staining of the cells was observed (Pic. 2)

Immunohistochemical staining of tissues of invasive mole, which is a malignant form of trophoblastic disease, showed a high expression of the CLIC1 protein in the nuclei of cytotrophoblasts in more than half of the cases.

The results of an immunohistochemical study of invasive mole showed that in 48% of cases there was a high expression of the CLIC1 protein in the nuclei of cytotrophoblasts. This indicates a high prognostic and diagnostic role of the CLIC1 protein.

Expression of the CLIC1 protein in a study of group 3 of 21 patients diagnosed with choriocarcinoma showed that in 18 samples (85.7%) strong staining of cytotrophoblast nuclei was observed, a negative result was not observed in any case, 5% have a moderately positive reaction and in 10% of cases a positive reaction (Pic. 3).

Immunohistochemical studies of preparations of 21 patients with choriocarcinoma showed a total of 96% positive reactions.

Immunohistochemical study of choriocarcinoma tissues showed that out of 21 histologically confirmed patients diagnosed with choriocarcinoma, 86% of patients had a high positive reaction.

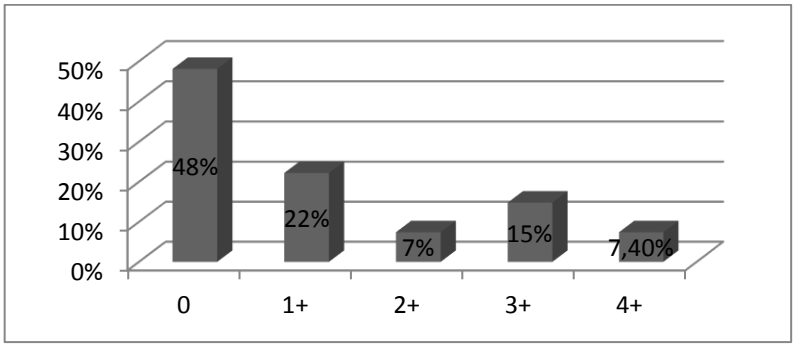
Immunohistochemical study of the CLIC1 antigen in 25 patients of the control group with a diagnosis of non-developing pregnancy showed the following results: 18 (72%) had no expression, 7 (28%) had weak staining. Moderate and strong cell staining was not detected (Pic. 4).

It is difficult to judge the true incidence of the disease, because not all cases of abortion are registered and the materials of curettage are not subjected to histological examination [2, 18]. This leads to a complicated course of pathology due to the lack of specific treatment and monitoring of patients. As a result, it is not rare to develop a malignant process, followed by hysterectomy in women of reproductive age [9, 15]. It is important to note the need for a required histological examination of the material obtained by the scraping of uterine cavity during a missed miscarriage and abortion. The necessity of these studies stems from the fact that there is an increasing number of cases when, in the absence of any clinical symptoms (due to early ultrasound examination), a complete HM is misdiagnosed as a missed miscarriage [19]. The importance of registration of all cases of HM is evidenced by the fact that in recent years the issue of the need for staging HM as stage 0 of GTD with subsequent restaging in the case of the development of a malignant trophoblastic tumor initiated by HM has been actively discussed [14].

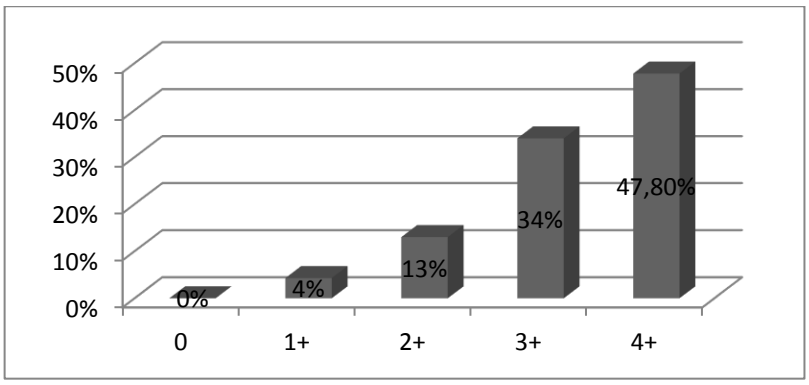
The study has shown that GTD is not a rare disease [20], as previously thought, especially its malignant forms.

Conclusions

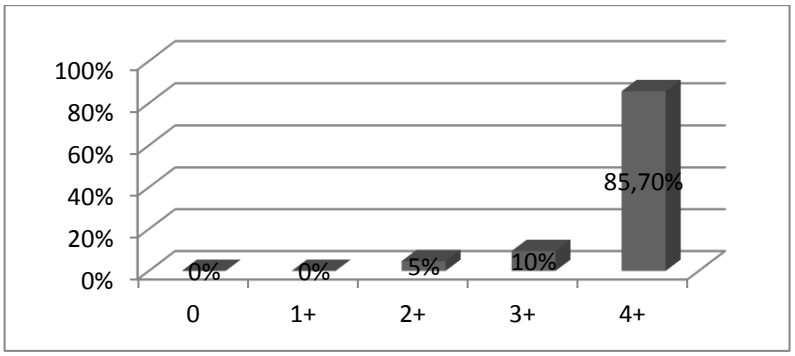
We observed higher levels of CLIC1 immunoreactivity in trophoblastic cells in patients with GTN compared with patients with HM. In the control group, the result was negative, indicating the absence of cells at risk of malignant transformation. The level of CLIC1 activity is increased in malignantly transformed cells of invasive mole and choriocarcinoma, being expressed in the nucleus and cytoplasm of trophoblastic cells. Thus, CLIC1 can serve as a prognostic marker that allows early detection of malignant transformation of HM. The results of the present study provide a basis for the development of prognostic markers that may help in the early prediction of GTN. More experiments are needed to validate CLIC1 as a biomarker and confirm its clinical efficacy.



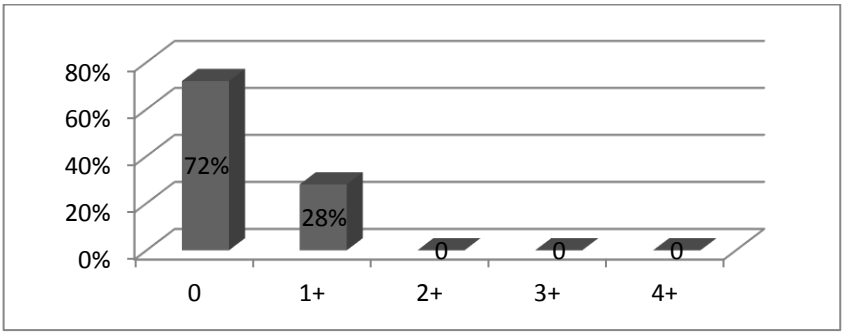
Picture 1. Distribution of patients diagnosed with hydatidiform mole depending on the severity of staining



Pic. 2. Distribution of patients diagnosed with invasive mole depending on the severity of staining.



Pic. 3. Distribution of patients with choriocarcinoma depending on the severity of staining



Pic. 4. Distribution of patients in the control group depending on the severity of staining

LIST OF REFERENCES:

1. M.R. Mamatova, N.N. Zaxirova, D.A. Nadjmitdinova. Gestatsion trofoblastik kasalligining turli shakllarining klinik-patologik xususiyatlari va tarqalishi // Tibbiyotda yangi kun jurnali. 4(32)2020 – C. 319-324
2. Berek J.S.. Berek & Novak's Gynecology, 14th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2007; 37: 1582–1604.
3. Behtash N., Hasanzadeh M., Hanjani P. Complete remission of an unusual location of metastatic GTN: A case report. Cancer Ther 2005; 2: 575–577.
4. Makovitzky J., Radtke A., Shabani N., Friese K., Gerber B., Mylonas I. Invasive hydatidiform mole: Immunohistochemical labelling of inhibin/activin subunits, Ki67, p53 and glycodelin A in a rare case. Acta Histochem 2009; 111:
5. 360–365.
6. Xue W.C., Khoo U.S., Ngan H.Y. et al. Minichromosome maintenance protein 7 expression in gestational trophoblastic disease: Correlation with Ki67, PCNA and clinicopathological parameters. Histopathology 2003; 43: 485–490.
7. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. Int J Gynecol Obstet 2002; 77: 285–287.
8. Menczer J., Schreiber L., Berger E., Golan A., Levy T. Assessment of Her-2/neu expression in hydatidiform moles for prediction of subsequent gestational trophoblastic neoplasia. Gynecol Oncol 2007; 104: 675–679.
9. Soper J.T. Staging and evaluation of gestational trophoblastic disease. Clin Obstet Gynecol 2003; 46: 570–578.
10. Yazaki-Sun S., Daher S., de Souza Ishigai M.M., Alves M.T., Mantovani T.M., Mattar R. Correlation of c-erbB-2 oncogene and p53 tumor suppressor gene with malignant transformation of hydatidiform mole. J Obstet Gynaecol Res 2006; 32: 265–272.
11. Friedli M., Guipponi M., Bertrand S., et al. Identification of a novel member of the CLIC family, CLIC6, mapping to 21q22.12. Gene. 2003;320:31Y40.
12. Chen C.D., Wang C.S., Huang Y.H., et al. Overexpression of CLCI1 in human gastric carcinoma and its clinicopathological significance. Proteomics. 2007;7:155Y167.

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