

EARLY PREDICTORS OF GESTATIONAL TROPHOBLASTIC NEOPLASIA

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Abstract. 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.

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

Introduction. 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 (CC). [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].

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-Cadherin, 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].

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

Materials and methods of research: For this study, 71 patients with GTD were selected and divided into 3 groups: 27 samples of HM, 23 - invasive mole, 21 - CC, 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 PD - 29.1 ± 3 , with IPD and HC - 32.3 ± 4 years.

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 diaminobenzidine (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.

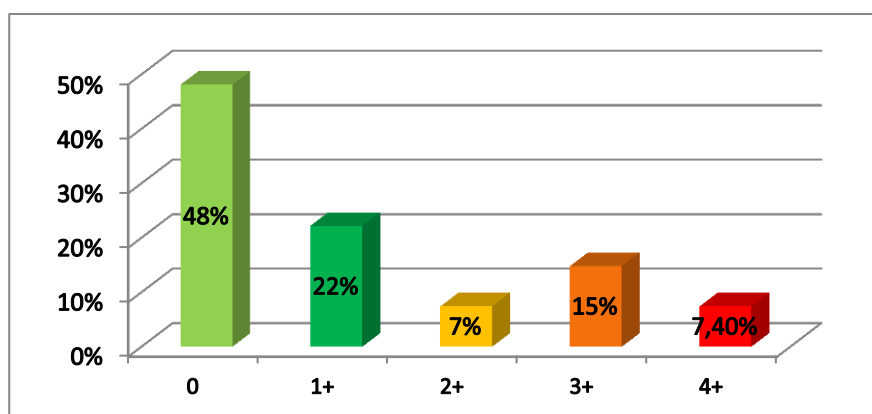
Sections were scored according to the percentage of CLIC1-stained trophoblast cells: 0 - for no positive cells, 1- for positive cells from 1% to 20%, 2- from 21% to 50%, 3 - from 51% to 80%, and 4 - from 81% to 100% of positive cells.

Results.

Table 1. Comparison of CLIC1 expression in the studying groups

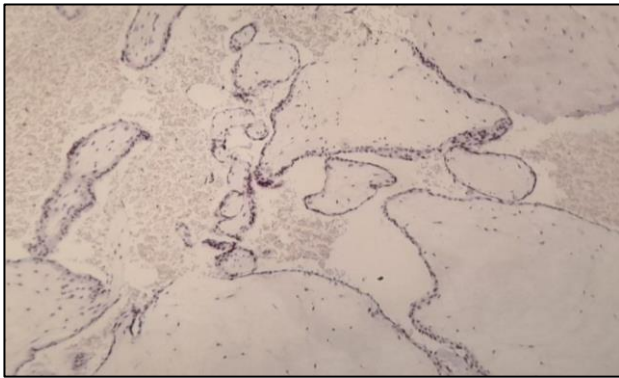
Groups	0 (no staining)	1+ (1 – 20%)	2+ (21 – 50%)	3+ (51 – 80%)	4+ (>50%)	N
Hydatidiform mole	13 (48,1%)	6 (22,2%)	2 (7,4%)	4 (14,8%)	2 (7,4%)	27
Invasive hydatidiform mole	0	1 (4,3%)	3 (13%)	8 (34,0%)	11 (47,8%)	23
Choriocarcinoma	0	0	1 (4,7%)	2 (9,5%)	18 (85,7%)	21
Control group	18 (72%)	7 (28%)	0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

Immunohistochemical study of the CLIC1 antigen in patients of group 1 diagnosed with hydatidiform mole showed the following results: out of 27 patients, 13 (48.1%) had no expression and 6 (22.2%) showed strong cell staining (Fig. 1).

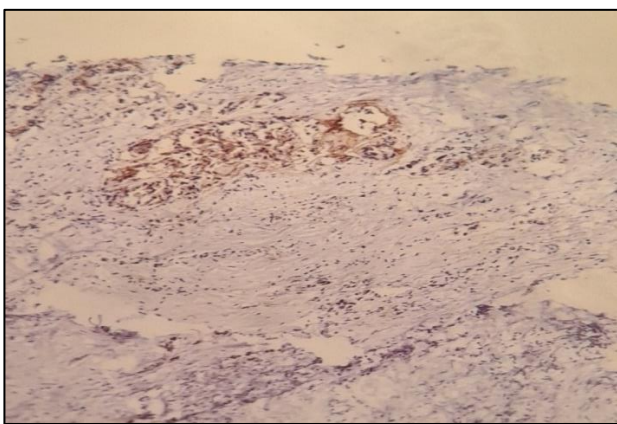


Picture 1. Distribution of patients diagnosed with hydatidiform mole depending on the severity of staining

As seen in Figure 2, the CLIC1 protein was weakly expressed in the cytoplasm and nuclei of some trophoblasts in most hydatidiform mole tissue samples.



Pic.2. HM. Immunohistochemical picture: CLIC1 protein - negative reaction.

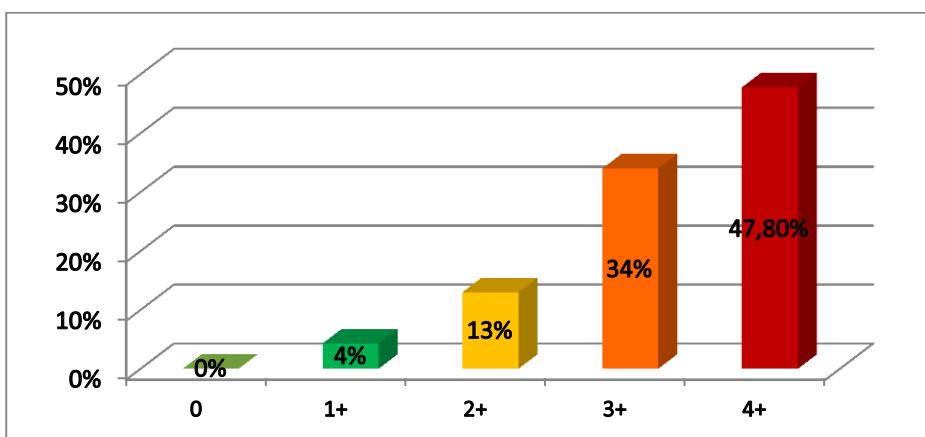


Pic. 3. HM. Immunohistochemical picture: CLIC1 protein - high positive reaction.

Picture 3 shows a staining of 4 points, which indicates a possible malignant transformation of the bladder tissue into choriocarcinoma.

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

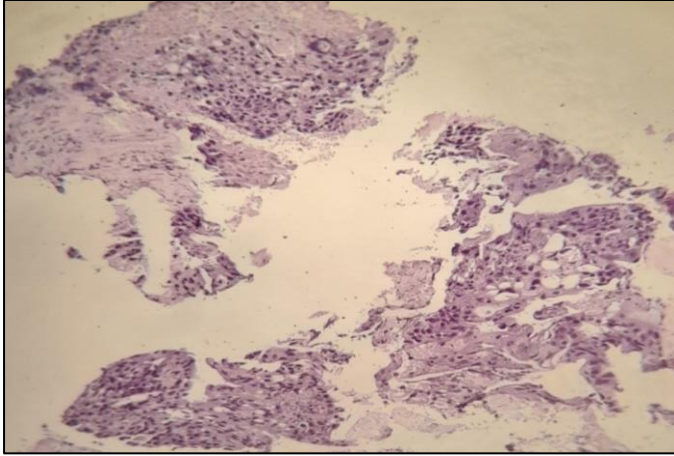
In group 2, in 23 patients with a histological diagnosis of invasive mole, the results of the IHC study showed that the absence of reactivity was not observed in any case and in 11 cases (47.8%) strong staining of the cells was observed (Pic. 4).



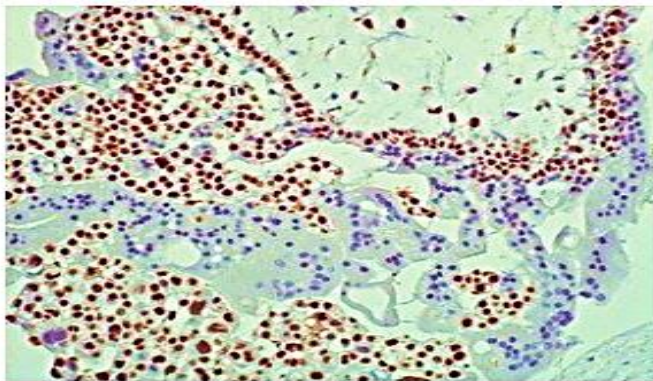
Pic. 4. Distribution of patients diagnosed with invasive mole depending on the severity of staining.

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 (Pic. 5).

Picture 6 shows a tissue sample from an invasive mole with a weak positive result.



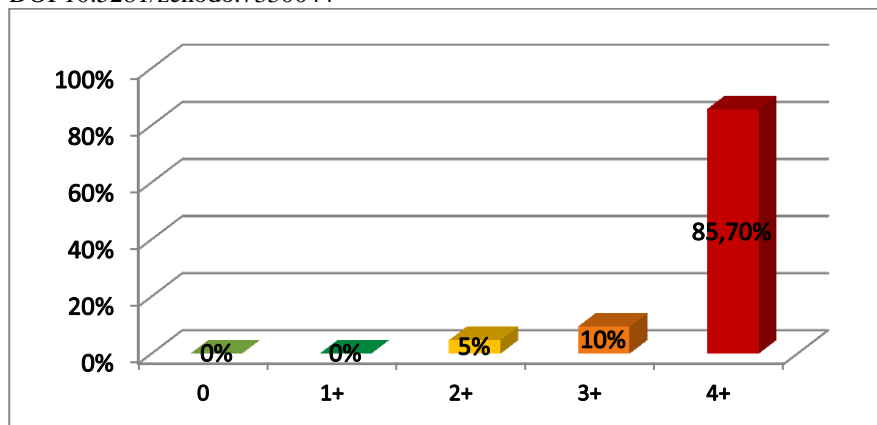
Pic. 5. Invasive hydatidiform mole. Immunohistochemical picture: Increased expression of the CLIC1 protein in the nuclei of some cytotrophoblasts.



Pic. 6. Invasive hydatidiform mole. Immunohistochemical picture: CLIC1 protein - high expression in the nuclei of cytotrophoblasts.

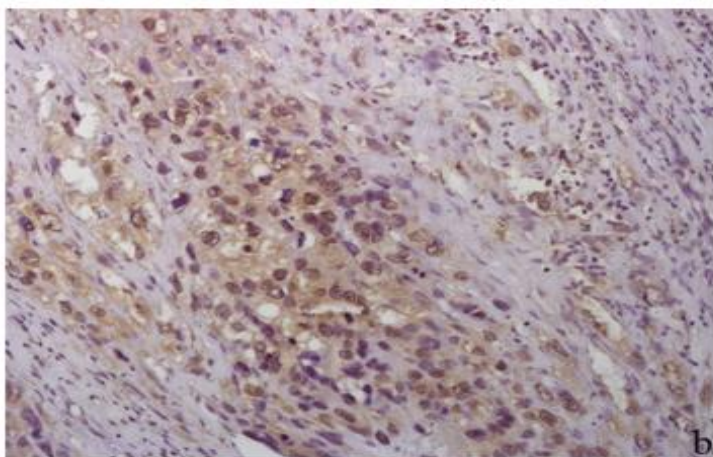
The results of an immunohistochemical study of invasive mole showed that in 48% of cases there was a high expression of the CLIC1 protein in the nuclei of cytotrophoblasts. This indicates a high prognostic and diagnostic role of the CLIC1 protein.

Expression of the CLIC1 protein in a study of group 3 of 21 patients diagnosed with CC showed that in 18 samples (85.7%) strong staining of cytotrophoblast nuclei was observed, a negative result was not observed in any case (Pic. 7).



Pic. 7. Distribution of patients with choriocarcinoma depending on the severity of staining

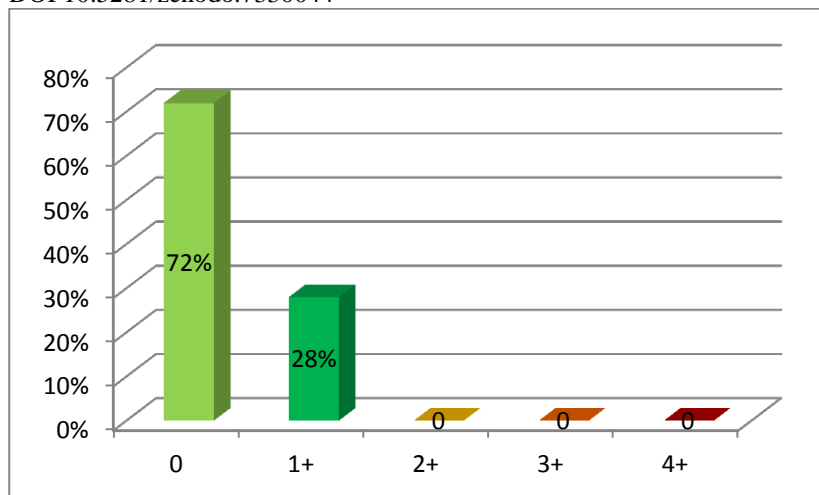
Immunohistochemical studies of preparations of 21 patients with choriocarcinoma showed a total of 96% positive reactions. (Picture 8.)



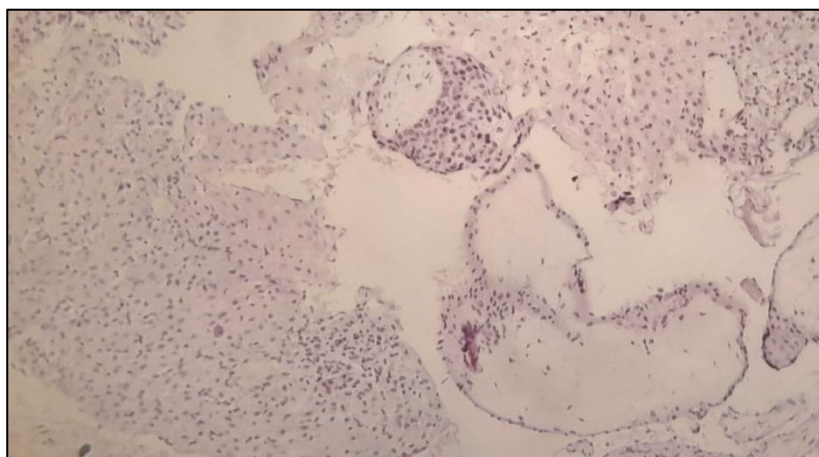
Pic. 8. Choriocarcinoma. Immunohistochemical picture: CLIC1 protein - high protein expression in the nuclei of almost all cytotrophoblasts.

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

IHC 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. 9).



Pic. 9. Distribution of patients in the control group depending on the severity of staining



Pic. 10. Non-developing pregnancy. Immunohistochemical picture: CLIC1 antigen - negative reaction.

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.

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 JS. Berek & Novak's Gynecology, 14th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2007; 37: 1582–1604.
2. Behtash N, Hasanzadeh M, Hanjani P. Complete remission of an unusual location of metastatic GTN: A case report. *Cancer Ther* 2005; 2: 575–577.
3. 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: 360–365.
4. Xue WC, Khoo US, Ngan HY et al. Minichromosome maintenance protein 7 expression in gestational trophoblastic disease: Correlation with Ki67, PCNA and clinicopathological parameters. *Histopathology* 2003; 43: 485–490.
5. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. *Int J Gynecol Obstet* 2002; 77: 285–287.
6. 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.
7. Soper JT. Staging and evaluation of gestational trophoblastic disease. *Clin Obstet Gynecol* 2003; 46: 570–578.
8. Yazaki-Sun S, Daher S, de Souza Ishigai MM, Alves MT, Mantovani TM, 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.
9. 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.
10. Chen CD, Wang CS, Huang YH, et al. Overexpression of CLCI1 in human gastric carcinoma and its clinicopathological significance. *Proteomics*. 2007;7:155Y167.