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ORIGINAL PAPERS

E-CADHERIN IMMUNOEXPRESSION IN COMPLETE AND PARTIAL HYDATIDIFORM MOLE

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ABSTRACT

Aim: The aim of the study was to estimate the correlation between E-cadherin immunoexpression and patohistological features of hydatidiform moles (HM) evacuated during the first trimester of pregnancy.
Material and methods: Research was performed on 100 samples of paraffin embedded with both 50 complete and 50 partial HM, with a confirmed diagnosis following a second pathologist review. Semi-quantitative analysis of the patohistological features of the mole (hydrops of villi, trophoblast proliferation, trophoblast pseudoinclusions, trophoblast nuclear atypia) was performed as well as E-cadherin immunostaining of representative slides. The pattern of E-cadherin immunoexpression was scored semi-quantitatively for intensity of staining and percentage of positive cells among villous cytotrophoblasts.
Results: Trophoblast pseudoinclusions, a common feature in partial but not complete moles, showed a strong positive correlation with E-cadherin expression intensity but not with the percentage of positive cells. Hydrops, trophoblast proliferation, and trophoblast nuclear atypia showed a strong negative correlation with E-cadherin expression, both in intensity of expression and percentage of positive cells.
Conclusion: Trophoblast pseudoinclusions in first trimester molar specimen, are a strong indicator of the benign potential of partial moles.
Key words: E-cadherin, immunohistochemistry, complete mole, partial mole

INTRODUCTION

E-cadherin (epithelial cadherin, CDH1, Cadherin 1) is a transmembrane Ca-dependent glycoprotein of great significance in maintaining cellular polarity and adhesion and improving benign, non-invasive epithelial growth and proliferation. In human embryogenesis, E-cadherin is described as a negative regulator of trophoblast growth and one of the most important factors of endometrial receptivity, blastocyst adhesion, and its implantation. Expression of E-Cadherin, starting at the two-cell stadium of mammalian development, reveals its fundamental role in postconceptional events [1–3].
Epigenetic modification is essential for trophoblast motility and invasion. The development of placental tissue in a disturbed ratio of maternal and paternal genes characterizes dynamic changes at the cellular and molecular levels [4–6].
Hydatidiform mole (HM), the most common form of gestational trophoblastic disease (GTD), develops from regular conception failure with an excess of paternal genes. Moles are benign trophoblast tumors with edematous placental tissue, presenting in complete (CHM) or partial (PHM) form. Gestational age strongly affects the presence of the typical pathohistological presentation of these two entities. Early and very early molar specimen usually presents [7–9].
E-cadherin was investigated in molar pregnancies [10,11], but the contribution of each morphology criteria to expression remains unknown.
Immunostaining for E-cadherin expression was performed in order to determine the relationship between pathohistological features of HM that contribute to the potential for uncontrolled trophoblast growth and invasion.

MATERIAL AND METHODS

Research was conducted on 100 molar specimens, signed as either complete (50) or partial (50), all evacuated during the first trimester by suction curettage. A second review of slides stained with hema-

toxylin and eosin by a single experienced pathologist was performed in order to confirm the diagnosis.

Patohistological analysis

Semi-quantitative analysis of samples was performed in order to estimate: hydrops of villi (focal or generalized) trophoblast proliferation (focal or diffuse), trophoblast pseudoinclusions (absent, round, irregular), and nuclear atypia in villous cytotrophoblast (absent, mild, moderate, strong).

Immunostaining

Following semiquantitative analysis, the selection of representative slides for E-cadherin immunostaining was performed. The immunohistochemistry staining procedure was performed on formalin-fixed, paraffin-embedded tissue samples cut at 4μm, using monoclonal rabbit antihuman antibody (clone 24E10, Cell Signaling Technology, Massachusetts, USA) with a 1:400 dilution. Prior to staining, 1mM citric buffer (pH 8.0 at 100°C, 10-minute duration) was used for antigen retrieval. The Immunostaining Center, Shandon Sequenza, was used for all incubation stages. After 30 minutes of incubation with the primary antibody, samples were treated with the secondary antibody (biotin, streptavidin, and peroxidases). The sections were counterstained with Mayer's hematoxylin, and Canada balsam was used for mounting the slides. Prostatic tissue applied to every slide and treated with the same procedure served as an external positive control. A Nikon ECLIPSE E400 microscope, with magnifications of 20x and 40x, was used for the analysis of E-cadherin expression.

E-cadherin positivity was defined as the presence of brown color immunoexpression of the cell membrane of the villous cytotrophoblast. Semi-quantitative analysis was used to estimate the percentage of positive cells: 0 <5%; 1 (5-15%); 2 (25-50%); 3 (50-75%); 4 >75%; and staining intensity was scored as follows: 0 – negative, 1 – weak, 2 – moderate, and 3 – strong intensity.

Statistical analysis One-factor multivariate analysis of variance (MANOVA) was used for statistical analysis and performed using the IBM SPSS software version 25. P values ≤ 0.05 were considered statistically significant.

RESULTS

The results of the semi-quantitative analysis of morphological criteria and the semi-quantitative analysis of E-cadherin expression for both complete and partial mole (expressed in percentage of positive samples) are summarized in **Table 1**. and **Table 2**.

Table 1. Morphological criteria in complete (CHM) and partial (PHM) mole

	Hydrops		Trophoblast proliferation		Trophoblast pseudoinclusions			Trophoblast atypia		
	Focal	Diffuse	Focal	Diffuse	Absent	Round	Irregular	Absent	Weak	Moderate
CHM (%)	6	94	2	98	74	2	24	6	28	36
PHM (%)	95	5	100	-	42	10	48	82	16	2

Table 2. The percentage of E-cadherin positive cells and E-cadherin staining intensity in complete and partial mole

	% E-cadherin positive cells					E-cadherin staining intensity		
	<5	5-25	25-50	50-75	>75	Negative	Weak	Moderate
CHM (%)	-	-	18,6	28	53,4	-	51,2	39,6
PHM (%)	-	-	-	9,3	90,7	-	4,6	44,2

Documented complete and partial mole slides and representative cases of E-cadherin expression are presented in **Figure 1**.

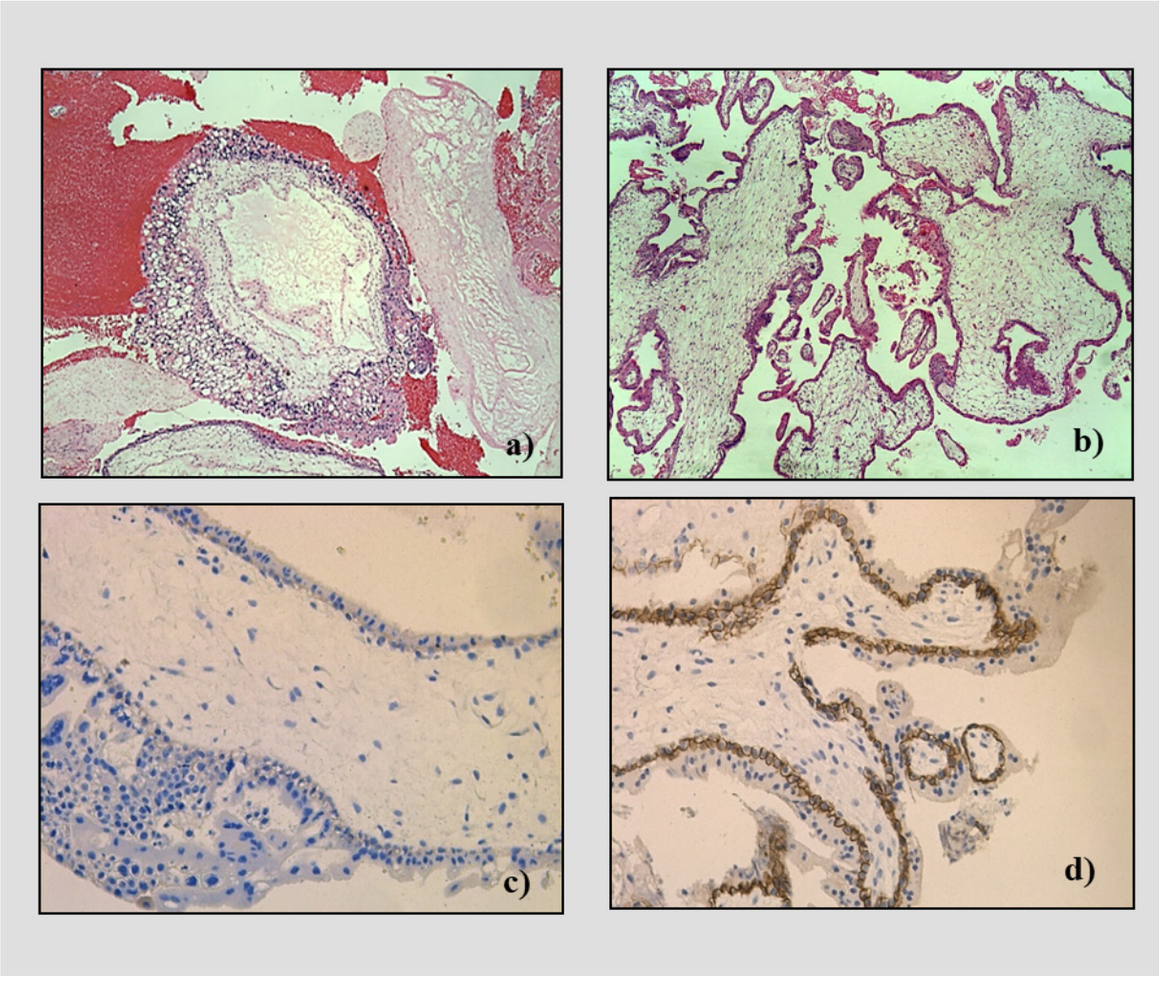

Figure 1. Typical presentation of the complete a) and partial b) mole [HE, x100]; the pattern of E-cadherin weak focal c), and strong diffuse d) expression in villous cytotrophoblasts [x200].

MANOVA test disclosed significant differences of single criteria influence on percentage of E-cadherin positive cells and staining intensity. Hydrops of villi, proliferation and atypia of the villous trophoblast show significant negative association with the percentage of E-cadherin positive cells. Staining intensity is in significant positive correlation with trophoblast pseudoinclusions, and a significant negative correlation with hydrops of villi, proliferation and trophoblast nuclear atypia ($p<0,05$, respectively) (Table 3). Trophoblast pseudoinclusions and percentage of positive cells was not in significant correlation.

Table 3. The correlation of the percentage of E-cadherin positive cells and staining intensity with morphology criteria

	E-cad %	E-cad intensity	Hydrops	Trophoblast proliferation	Trophoblast pseudoinclusions	Atypia
E-cad %	1	0,72**	-0,35**	-0,43**	0,19	-0,45**
E-cad intensity	0,72**	1	-0,43**	-0,54**	0,31**	-0,49**
Hydrops	-0,35**	-0,43**	1	0,81**	-0,00	0,64**
Trophoblast proliferation	-0,43**	-0,54**	0,81**	1	-0,25*	0,82**
Trophoblast pseudoinclusions	0,19	0,31**	-0,00	-0,25*	1	-0,14
Atypia	-0,45**	-0,49**	0,64**	0,82**	-0,14	1

** $p<0,05$

DISCUSSION

In this research, we performed an analysis of the first trimester molar specimen and the association of patohistological criteria and E-cadherin immunostaining outcome (% of positive cells and staining intensity). Villous hydrops and trophoblast proliferation demonstrate clear differences between complete and partial mole. Irregular trophoblast pseudoinclusions are present in every second sample of PHM, and in CHM similar findings are present in 25% of specimens. Nuclear atypia in villous trophoblast is inherent in most complete mole cases, but in partial mole is a relatively rare finding. Complete moles presents with more heterogenous E-cadherin expression, compared with partial mole. We found that trophoblast pseudoniclusions are in strong positive correlation with E-cadherin staining intensity but not a percentage of positive cells. Hydrops of villous stroma, trophoblast proliferation and atypia disclosed a significant negative correlation with both E-cadherin staining outcomes.

Although described as relatively rare pregnancy disorders, mechanisms of developing of hydatidiform mole are well known. Absolute (CHM) or relative (PHM) excess of paternal genes underlie florid trophoblast proliferation. Hydropic swelling, heterogenous population of villi and trophoblast nuclear atypia [12–14] serve as additional criteria for final diagnosis of CHM and PHM [15].

Villous trophoblast pseudonclusions in molar specimen develops from irregular trophoblast growth, with significant downfolding of epithelia into the villous stroma. Folding of epithelia can be forced with increasing local compression [16], which develops in the avascular villous stroma of molar specimen. Similar to our results, irregular villous contours and scalloped villi with the inclusion of trophoblast into the edematous stroma of villi are consistent with the diagnosis of partial but not complete mole [17,18].

First-trimester HM usually presents with poorly developed pathological features, posing a challenge for the differential diagnosis among pregnancy specimens with hydropic changes and trophoblast proliferation [7], [12].

Reports considering intra- and interobserver variability more closely describes challenges in the routine diagnostic approach [19,20]. Second pathologist review in our research disclosed two nonomolar specimen with the diagnosis of PHM, which were substituted with two confirmed partial moles. Significant differences in postevacuational management and follow up between complete and partial HM require several ancillary techniques to ensure the exact distinguishing of these entities with overlapping pathohistological features [21–23].

Disturbed paternal to maternal genes ratio contributes to the level and pattern of expression of several products of genes following conception [24–26]. The complex role of E cadherin involved in epithelial architecture due to cell adhesion, contractile protein interaction and polarity is improved for both mono- and multi-layer epithelia [27]. E-cadherin is recognized as a reliable marker of epithelia-to-mesenchyme transition (EMT), molecular and phenotype changes of epithelial cells that enhance the invasive phenotype [28,29], with improved significance in early placental development [1,2].

E-cadherin expression is investigated in a wide spectrum of placental maldevelopment and results indicate that not only genetic disorders underlie changes in E-cadherin expression, significant changes were documented in acquired metabolic disorders. E-cadherin expression gradually decreased, with significant differences, from normal early trophoblastic tissue to complete mole and invasive mole [10], [30]. Significant but divergent changes in E-cadherin expression are described in mature placenta from pregnancies complicated with gestational diabetes and pregnancy-induced hypertension and preeclampsia in comparison with healthy placentas [31,32]. However, none of the studies more closely describes the correlation of pathological criteria for the diagnosis of complete or partial mole and the frequency or intensity of E-cadherin staining was not, as in our research. Yet, the studies investigating aneuploid conceptions, find that trisomy 16 is associated with low trophoblast proliferation and invasion but strong E-cadherin expression [33].

E-cadherin expression findings demonstrate some unclear results, with conflicting results in the same type of specimen. A study over more than 1000 samples of cancer of different types disclosed that E-cadherin expression is more likely to be either very low or very high. Neo-expression of E-cadherin is described as rare event, and low level of neo-expression is observed among cancer cells that derive from E-cadherin negative normal cells [34].

E-cadherin expression is reduced or even may be absent in some cases of disturbed epithelial growth. Heterogenous membranous and in some cases cytoplasmic expression is observed among same type of cancer, and moreover, positivity in some cases was associated with unfavorable tumor outcome [34–38] In our molar specimen cytoplasmic expression was not detected. Earlier findings of divergent immunoexpression detects several distinct causes (clone antibody dilution and detection of chromogen, tissue sample, level of antigen expression). Simple detection of positive or negative cell/membrane/cytoplasm is easy to perform. Though the grading of staining intensity might suffer from lack the objectivity, especially in extreme intensity staining results, semi-quantitative analysis still represents the reliable diagnostic tool in immunostaining procedure [39–41].

CONCLUSION

Complete moles presented with more heterogenous percentage of E-cadherin positive cells, compared with partial mole. Unlike trophoblast pseudoinclusions, a significant negative correlation of E-cadherin immunoexpression was disclosed for hydropic villi, tropho-

blast proliferation and trophoblast nuclear atypia. The presence of trophoblast pseudoinclusions is a strong indicator of the benign potential of partial mole.

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TRANSPARENCY DECLARATION

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