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RESEARCH ARTICLE

Analysis of p53 expression in partial hydatidiform mole and hydropic abortion

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BACKGROUND: Gestational trophoblastic disease (GTD) is a heterogeneous group of disorders characterized by abnormal trophoblast tissue. Molar and non-molar hydropic placental changes are the most common forms of GTD. Differential diagnosis of GTD is sometimes problematic. Recently, p53 expression was identified as a good marker for distinguishing GTD types.
AIMS: Comparison of p53 expression in partial hydatidiform mole (PHM) and hydropic abortion.
METHODS: In this prospective cross-sectional study, molar and non-molar hydropic pregnancy specimens were collected. Immunohistochemical staining, based on the Labeled Streptavidin Biotin (LSAB) technique, was carried out on multiple 4 μm paraffin block sections prepared from formalin-fixed trophoblastic tissues. Polymer-based Envision was used to assess p53 tumor suppressor protein immunoreactivity. p53 expression was then compared between both groups.
RESULTS: In the study, 40 patients were included: 20 with confirmed PHM and 20 with hydropic pregnancy. p53 protein was positive in 60% of patients with PHM and 25% of patients with hydropic pregnancy. The p53 positive rate was significantly higher in patients with PHM ($p = 0.027$). Moreover, patients with PHM had a significantly high grade of staining ($p < 0.001$).
CONCLUSION: Our findings indicate that immunohistochemical analysis of p53 protein can be used to distinguish PHM and hydropic pregnancy.

Keywords Partial hydatidiform mole, hydropic abortion, p53 expression

Introduction

A variety of cellular stressors, such as DNA damage, are detrimental to human cells and ultimately result in genomic instability. Accumulation of different genetic abnormalities leads to cancer. Thus, an appropriate response is needed to prevent cells from acquiring neoplastic transformations. p53 protein is encoded by the *TP53* gene, located at 17p13.1. It acts as a tumor suppressor and responds to different cellular stress signals by regulating gene expression. p53 induces cell cycle arrest, apoptosis, senescence, DNA repair, and/or metabolic modifications within the cell (Berchuck et al., 1994). It is an important tumor suppressor and the most frequently disrupted protein in human cancers (Ozaki and Nakagawara, 2011). One of the most studied p53 functions is apoptosis, which was first reported in irradiated mouse thymocytes by Clarke and Lowe in 1993. p53-dependent apoptosis has since been studied in a wide range of cell types, and in response to different stress signals. When the cell is triggered by a stress signal, p53 induces transcription of apoptotic target genes (Soragni et al., 2016). Dysregulation of apoptosis has been reported in many pathological conditions, including human malignancies. Recent studies have shown that apoptosis plays an important role in the pathogenesis of gestational trophoblastic disease (GTD) (Chiu et al., 2001).

GTD is a heterogeneous group of diseases characterized by abnormal trophoblast tissue. The most common form of GTD is molar pregnancy. It is widely distributed across different parts of the world, with a particularly high incidence rate in Asian countries (Singh et al., 2016). The prevalence of molar pregnancy in Iran is estimated at 7 per 1000 pregnancies (Almasi et al., 2014). Molar pregnancy is divided into two major groups, including complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). Molar pregnancy is usually diagnosed by ultra-sonography, human chorionic gonadotropin (hCG) elevation, and clinical presentation (Ngan et al., 2015). Accurate diagnosis and differentiation of hydatidiform moles is very important because of the risk of progression to malignancy. Thus, non-molar placenta and

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hydropic abortion must be distinguished from CHM and PHM. Regarding the limitations of microscopic examination, complementary methods such as immunohistochemical (IHC) and genetic markers have been introduced to facilitate more accurate differential diagnosis of molar and non-molar placental disorders (Carey et al., 2015; Masood et al., 2015).

The role of apoptosis in GTD may reflect changes in the expression of apoptosis regulator genes. As stated above, p53 is one of the key regulators of apoptosis. It was thus hypothesized that its expression may be altered in GTD. Many studies support this hypothesis, showing high p53 expression in CHM and PHM (Rath et al., 2011; Hasanzadeh et al., 2016). Hence, we aimed to compare p53 expression in CHM and hydropic abortion.

Material and methods

Study population

In this prospective cross-sectional study, patients with a confirmed molar pregnancy diagnosis that were referred to the pathology department of Imam Khomeini hospital, Ahvaz, Iran between 2010 and 2014 were included. Formalin-fixed, paraffin-embedded specimens were collected and stained with routine hematoxylin-eosin and histopathologically reviewed by two independent expert pathologists, based on criteria for diagnostic confirmation (Ngan et al., 2015). Patient demographic information was recorded from patient files. The study was approved by the ethics committee of Ahvaz Jundishapopr University of Medical Sciences. The study protocol was explained to all participants prior to obtaining their written informed consent.

IHC evaluation

IHC staining, based on the Labeled Streptavidin Biotin (LSAB) technique (http://www.ihcworld.com/_ protocols/general_IHC/standard_lsab_method.htm), was carried out on multiple 4 μm paraffin block sections prepared from formalin-fixed trophoblastic tissues. The sections were dehydrated and then incubated in a microwave for 15 min. Then, H_2O_2 in 0.03% methanol was added for 10 min. Monoclonal p53 antibody, diluted 1:100, was added overnight. The samples were placed in phosphate buffer saline (PBS) (pH 7.4) and incubated with polymer-based Envision to assess p53 immunoreactivity. PBS was assessed with antibodies as a negative control. All slides were reviewed by two expert pathologists under a light microscope (OlympusB × 50; Olympus optical Co, Ltd, Tokyo, Japan). At least 100 trophoblastic cells were evaluated in each section. The percentage of nuclei in each section was determined. The rate of p53 expression was reported as the percentage of cytotrophoblastic and syncytiotrophoblastic cells with positive nuclear immunoreactivity. Tumors with $> 5\%$ p53-positive cells were considered to be p53 positive tumors.

Statistical analysis

To compare the 2 groups, a *t*-test was used. The comparison of qualitative variables between the 2 groups was done using the chi square and the Fisher exact test. In all the analyses, $p < 0.05$ was defined as the level of significance. The data were analyzed using SPSS 21.0.

Results

In the study, 40 patients were included: 20 with confirmed PHM and 20 with hydropic pregnancy. The mean age of patients with PHM and hydropic pregnancy were 27.5 and 28.6 years old, respectively, which was not significantly different. The parity number in the PHM and hydropic pregnant women were 2.4 and 2.05, respectively. Table 1 shows patient characteristics.

Table 1 Patient characteristics in both groups.

Variables	Partial mole $n = 20$	Hydropic $n = 20$	p value
Age	27.5±6.9	28.6±8.4	$p = 0.65$
Parity number	2.4±1.09	2.05±1.09	$p = 0.31$
p53 status			
Yes	12 (60%)	5 (25%)	$p = 0.027$
No	8 (40%)	15 (75%)	

p53 protein was positive in 60% of patients with PHM and 25% of hydropic. The p53 positive rate was significantly higher in patients with PHM ($p = 0.027$). Moreover, the grade of staining was compared in patients with and without abortion history and it was shown that patients with abortion history had a significantly high grade of staining ($p = 0.005$). The same results were also observed in patients with PHM,

Table 2 Relations between grade of staining and clinical findings.

Variables		Grade of staining				p value
		Negative (0)	1	2	3	
Abortion history	Yes	2 (28.6%)	2 (28.6%)	3 (42.9%)	0 (0%)	$p = 0.005$
	No	21 (63.6%)	11 (33.3%)	1 (3%)	0 (0%)	
GTD	Partial mole $n = 20$	8 (40%)	9 (45%)	3 (15%)	0 (0%)	$p < 0.0001$
	Hydropic $n = 20$	15 (75%)	4 (20%)	1 (5%)	0 (0%)	

i.e., they had significantly high grade of staining ($p < 0.001$) (Table 2).

Discussion

Progression of GTD toward gestational trophoblastic neoplasia is a major gynecological concern. Different GTD types have a wide-ranging risk of malignant transformation. To date, differential diagnosis has been based on ultrasound evaluation and b-HCG assessment. Recently, investigators have attempted to evaluate genetic factors in GTD diagnosis. In the present study, p53 expression was immunohistochemically assessed in 20 patients with PHM and 20 patients with hydropic pregnancy. p53 expression was then compared between both groups.

Our findings showed that p53 expression is significantly higher in PHM than in hydropic pregnancy. Moreover, patients with PHM showed a significantly high grade of staining. These findings corroborate those of previous studies. Al-Bozom compared the p53 expression in 40 patients with molar (PHM and CHM) and non-molar hydropic changes. He showed that 93% of CHM, 57% of PHM and none of the patients with hydropic placentas had p53 expression (Al-Bozom, 2000). Chen *et al.* immunohistochemically evaluated p53 expression for differential diagnosis of hydropic abortion, PHM and CHM. They reported no observable p53 expression in normal placenta, and observable p53 expression in only 1 of 12 hydropic abortion cases. p53-positive rates in PHM and CHM were 60.9% (14/23) and 85.0% (17/20), respectively. They were significantly higher in PHM than in hydropic abortion, and significantly differed between PHM and CHM (Chen et al., 2011). However, the findings of Schammel *et al.'s* study were contrary to our findings. They immunohistochemically evaluated p53 expression in 60 molar and non-molar placentas with hydropic changes and showed that p53 expression did not significantly differ between the two groups (Schammel and Bocklage, 1996). In the previously mentioned study, the investigators assessed p53 expression in S-phase fractions derived from flow cytometric analysis, whereas we evaluated it using the LSAB technique on the tissue biopsy.

Chiu *et al.* reported that dysregulated apoptosis has the most important role in GTD pathogenesis, especially in molar pregnancy (Chiu et al., 2001). Moreover, Wei *et al.* showed that high p53 expression is linked with apoptotic level in the placental villus tissues (Wei et al., 2014). Thus, the high p53 expression in PHM and CHM can be explained by previous findings.

Collectively, our findings indicate that IHC analysis of p53 can be used to distinguish PHM and hydropic pregnancy. We did not analyze the association between p53 expression level and apoptosis in the molar and non-molar hydropic placenta change, and this is the limitation of our study.

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Compliance with ethics guidelines

The authors declare that they have no conflict of interest. All procedures have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down by the 1964 Declaration of Helsinki and its later amendments. Informed consent was assessed prior to intervention. Details disclosing the identity of the subjects under study were omitted

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