Expressions of p53, proliferating cell nuclear antigen, and Ki-67 in gestational trophoblastic diseases

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Summary

Objective: This study was done to determine whether the expressions of p53, PCNA, and Ki-67 could differentiate spontaneous abortions with hydropic changes from gestational trophoblastic diseases.

Materials and Methods: Twenty partial hydatidiform moles, 21 complete hydatidiform moles, nine invasive hydatidiform moles, three choriocarcinomas and 19 first trimester hydropic spontaneous abortions were evaluated by means of immunohistochemical methods with antibodies to p53, PCNA, and Ki-67 in this study.

Results: The Ki-67, PCNA, and p53 immunoreactivity was significantly higher in the gestational trophoblastic disease group than in the spontaneous abortion group with hydropic changes. None of the three parameters provided reliable discrimination among gestational trophoblastic disease subgroups.

Conclusion: Our findings suggest that expressions of Ki-67, proliferating cell nuclear antigen and p53 can be used to differentiate between spontaneous abortion with hydropic changes and gestational trophoblastic disease when all three markers are used together.

Key words: Gestational Trophoblastic Disease; Hydatidiform Mole; PCNA; Ki-67; p53.

Introduction

Gestational trophoblastic diseases are characterized by abnormal proliferation of the trophoblast and classified as partial hydatidiform moles, complete hydatidiform moles, invasive hydatidiform moles, choriocarcinomas, and placental-site trophoblastic tumors. Gestational trophoblastic diseases can be encountered following normal births, spontaneous abortions, ectopic pregnancies and even during pregnancy [1].

However, there is usually no difficulty in diagnosing choriocarcinomas and placental-site trophoblastic tumors, whereas distinguishing between spontaneous abortion with hydropic changes and partial hydatidiform mole or other forms of gestational trophoblastic disease remains a problem in spite of well-described histopathologic criteria [2]. Accurately distinguishing a molar gestation from a nonmolar hydropic abortion is important because of the former's propensity for persistent gestational trophoblastic disease and increased risk of subsequent choriocarcinoma [3].

Tumor cell kinetics directly influence the clinical outcome in cancer, and the identification and quantification of proliferative activity in neoplasms appears to be of both diagnostic and prognostic value. It is possible to determine the proliferative potential of cell populations in tumors by means of immunohistochemical methods [4].

Proliferating cell nuclear antigen (PCNA) and Ki-67 have been established as a valuable reflection of the tissue proliferative compartment and thus could be of value in studying the biologic behavior of molar gestations [5]. The p53 gene encodes a nuclear phosphoprotein. The p53 protein functions as a tumor suppressor, which negatively regulates cell growth. The nuclear phosphoprotein is present in extremely small amounts of normal tissue and is almost undetectable. However, mutations in this gene are the most common genetic alteration in human cancers and can be detected by immunohistochemical methods [6]. For these reasons, we decided to investigate the PCNA, Ki-67 and p53 protein in spontaneous abortion with hydropic changes as well as cases of gestational trophoblastic disease.

The aim of this study was to evaluate the expressions of Ki-67, PCNA and p53 in gestational trophoblastic disease and also to assess the values of these markers both individually and as a group in the differential diagnosis of gestational trophoblastic disease subgroups and spontaneous abortion with hydropic changes.

Materials and Methods

Patients. Twenty partial hydatidiform moles, 21 complete hydatidiform moles, nine invasive hydatidiform moles, 19 first trimester hydropic spontaneous abortions and three choriocarcinomas diagnosed previously in the Department of Pathology, School of Medicine, Dicle University, Diyarbakır, Turkey, were included in this study. The histolopathologic features of the specimens were evaluated according to the diagnostic criteria of Szulman [7, 8].

The median age of the patients was 25 years (range, 17-40 years) in the partial hydatidiform mole group, 29 years (range, 18-42 years) in the complete hydatidiform mole group, 27 years (range, 21-50 years) in the invasive hydatidiform mole group, and 25 years (range, 18-40 years) in the spontaneous abortion group. The ages of the three choriocarcinoma cases were 23, 29 and 35.

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Immunohistochemical studies. Multiple 6-µm-thick sections of representative formalin-fixed, paraffin-embedded tissues were cut for immunohistochemical studies. A streptavidin-biotin-peroxidase technique (Zymed Laboratories Inc, San Francisco CA) was used for the detection of Ki-67 (prediluted MIBI; Avezzona, Italy), PCNA (prediluted PC10), and p53 (1:50; DO7; Zymed Laboratories) with the antigen retrieval microwave method (BioGenex Laboratories, San Ramon, CA).

All immunostained sections were examined by the same two pathologists with a x400 magnification under a light microscope (Olympus BH-2; Olympus Optical Co, Ltd, Tokyo, Japan) for evaluation of Ki-67, PCNA, and p53 expression.

In the abortion specimens, partial hydatidiform moles, complete hydatidiform moles, invasive hydatidiform moles and choriocarcinomas, 1,000 villous cytotrophoblastic cells were counted in each case for Ki-67 and PCNA expression. All stained nuclei were scored as positive, regardless of staining intensity. The nuclear labeling index values for Ki-67 and PCNA immunoreactivity were determined by scoring positive nuclei per total number of counted nuclei for 1,000 cytotrophoblastic cells in each case. The p53 expression was determined by evaluating the percentage of positivity observed in the cytotrophoblastic cell population within placental tissue. Tissue sections of three choriocarcinoma cases were used to observe the staining intensity but they were not included in statistical analyses because there were only three cases.

Statistical analysis. The results obtained from the gestational trophoblastic disease group and spontaneous abortion group with hydropic changes were compared for all the parameters included in the study (Ki-67, PCNA and p53 expression) by means of the Mann-Whitney U test (SPSS 10.0 statistic program) and variance analysis. The results are expressed as median.

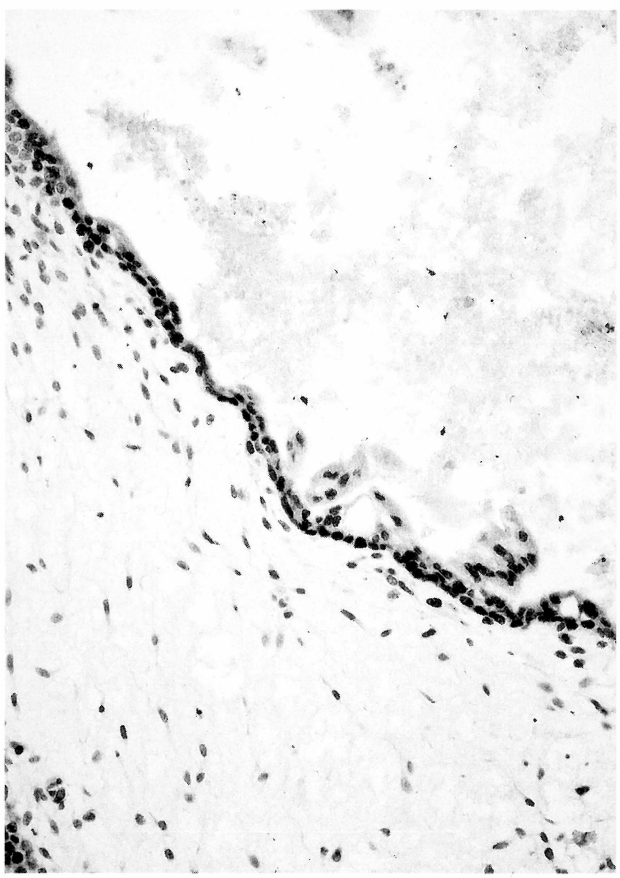

Figure 1. — PCNA positivity in trophoblastic cells of a spontaneous abortion with hydropic change case.

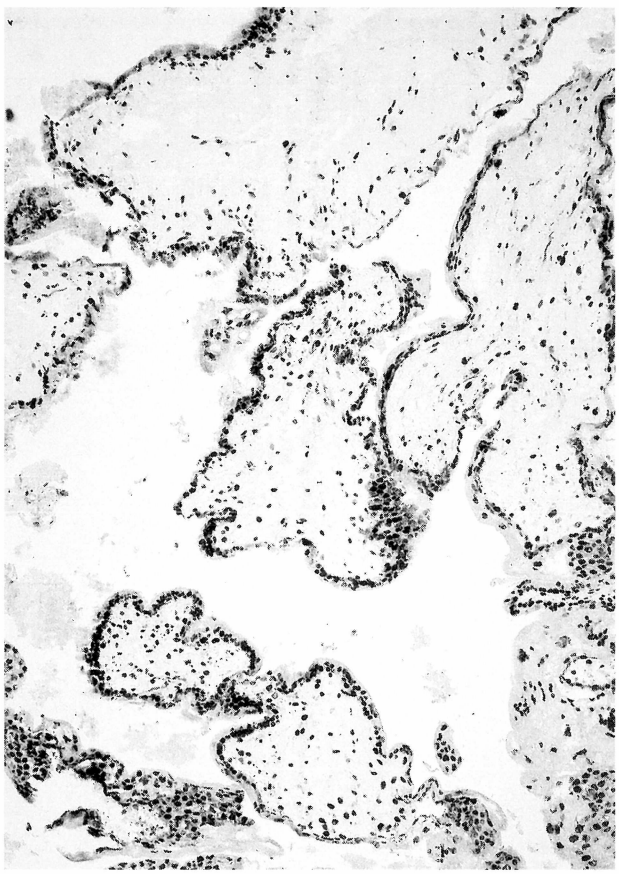

Figure 2. — Ki-67 positivity in trophoblastic cells of a partial hydatidiform mole case.

Results

Ki-67, PCNA, and p53 immunoreactivity were limited to cytotrophoblastic cell nuclei in nearly all cases, but in a few cases syncytiotrophoblastic cells had immunoreactivity.

The highest Ki-67, PCNA and p53 expressions were observed in choriocarcinomas and invasive hydatidiform moles, followed by complete hydatidiform moles and partial hydatidiform moles (Table 1) (Figures 1-5). Spontaneous abortions with hydropic changes had the lowest immunoreactivity for Ki-67, PCNA, and p53 (Table 2). There was also a statistically significant difference between the spontaneous abortion group with hydropic changes and the gestational trophoblastic disease group for all three parameters studied ($p < 0.001$).

A significant difference in p53 expression was observed between the spontaneous abortion group with hydropic changes and the gestational trophoblastic disease group ($p < 0.001$). None of those three parameters provided reliable discrimination among the gestational tropho-

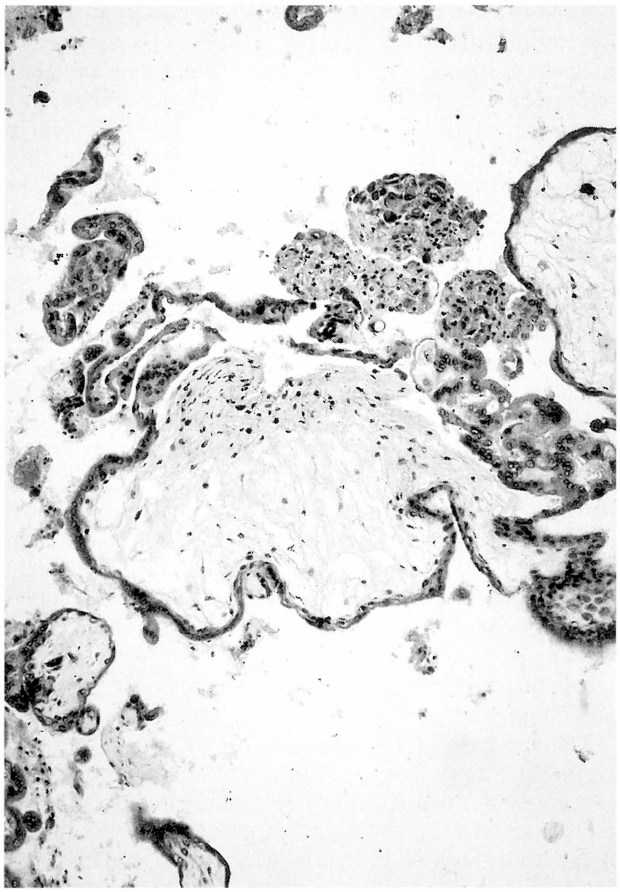

Figure 3. — PCNA positivity in trophoblastic cells of a complete hydatidiform mole case.

Table 1. — *Ki-67, PCNA and p53 values in spontaneous abortions with hydropic changes and gestational trophoblastic diseases.*

	Ki-67 (count/1000 cells)	PCNA (count/1000 cells	p53 (% positivity)
Spontaneous abortion with hydropic changes (n=19)	25	40	2.50
Partial hydatidiform mole (n=20)	200	250	20
Complete hydatidiform mole (n=21)	532	610	45
Invasive hydatidiform mole (n=9)	650	790	50
Choriocarcinoma (n=3)	895	955	65

Table 2. — *Parameters in gestational trophoblastic diseases and spontaneous abortions.*

	Ki-67 (count/1000 cells)	PCNA (count/1000 cells	p53 (% positivity)
Spontaneous abortion group with hydropic changes (n=19)	25	40	2,50
Gestational trophoblastic disease group (n=53)	503	551	25
Statistical significance	$p < 0.001$	$p < 0.001$	$p < 0.001$

All values are expressed as median.

Table 3. — *Sensitivity and specificity of each parameter.*

	Sensitivity (%)	Specificity (%)
Ki-67	88.19	100
PCNA	89.31	100
p53	67.85	100
All variables	100	100

blastic disease subgroups. However, sensitivity and specificity for discriminating gestational trophoblastic disease from spontaneous abortion with hydropic changes were 100% when all three markers were used together (Table 3).

Discussion

Hydatidiform moles have been divided into complete and partial forms based on morphologic, cytogenetic, and clinical features. When there are molar villi with associated trophoblastic cells in the myometrium and broad ligament or at distant sites, the pathologic diagnosis is invasive hydatidiform mole [1]. It is usually easy for the pathologist to differentiate between them and spontaneous abortion with hydropic changes; however, in some cases it may be difficult, particularly in the absence of diploidy studies [9].

This immunohistochemical study was designated to determine whether Ki-67, PCNA and p53 protein over-

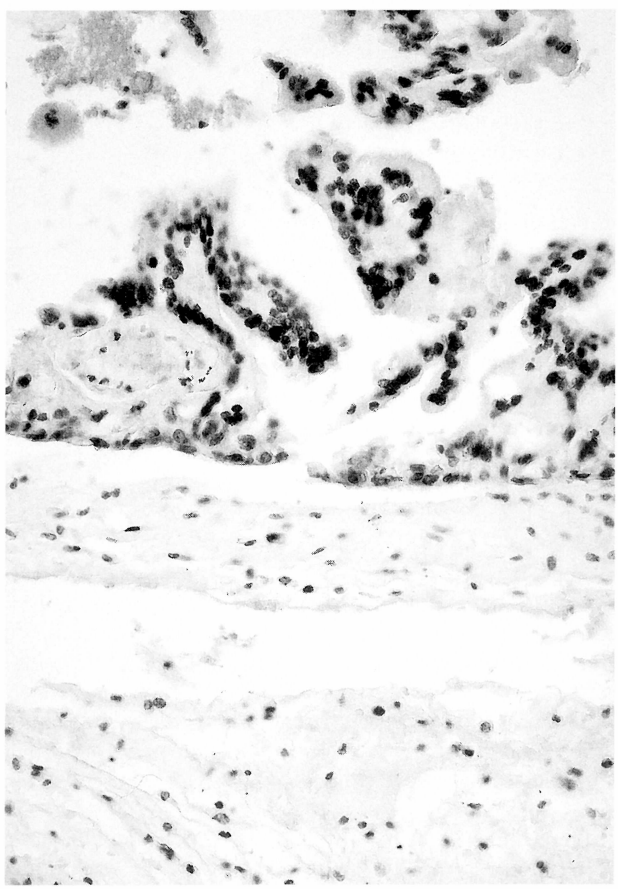

Figure 4. — p53 positivity in trophoblastic cells of a complete hydatidiform mole case.

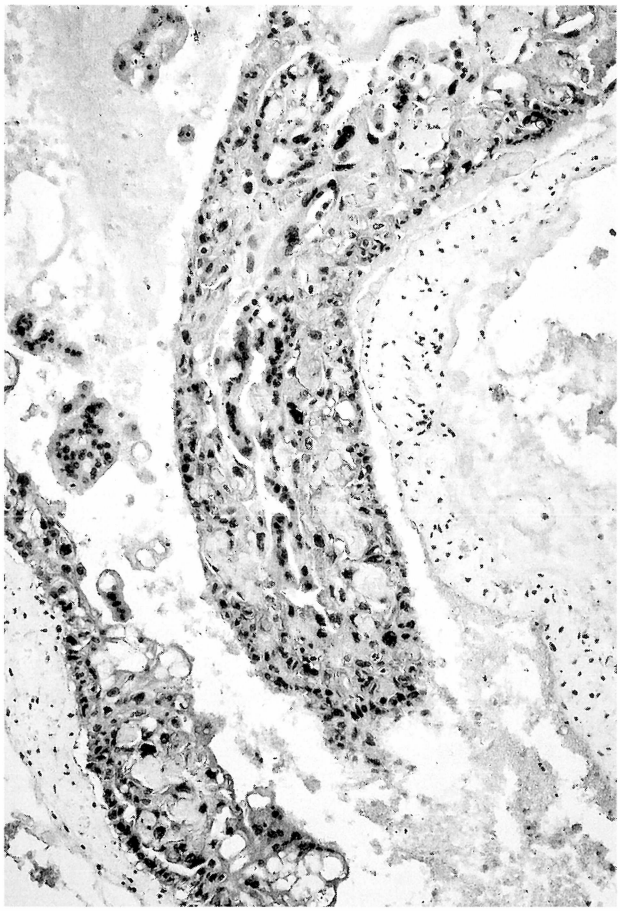

Figure 5. — Ki-67 positivity in trophoblastic cells of an invasive hydatidiform mole case.

expression in trophoblastic tissue would have any discriminatory value for spontaneous abortion with hydropic changes and gestational trophoblastic disease.

The median counts of cytotrophoblastic cells in spontaneous abortions with hydropic changes staining positively for Ki-67, PCNA, and p53 were much lower than those in gestational trophoblastic disease. This significant difference in the immunoreactivity of markers between spontaneous abortion with hydropic changes and gestational trophoblastic disease is probably caused by the trophoblastic proliferation of hydatidiform moles [10].

The highest expressions of Ki-67 and PCNA were observed in invasive hydatidiform moles. This result demonstrates that, in invasive moles, trophoblastic hyperplasia and proliferative activity are more pronounced than in partial, complete hydatidiform moles and spontaneous abortions with hydropic changes.

The p53 tumor suppressor gene is located on chromosome 17 and encodes for a nuclear phosphoprotein that binds to DNA, preventing progression of the cell from the G1 to the S-phase in the cell cycle. Wild-type p53 protein has a short half-life and is usually undetectable. Alterations in the p53 gene result in p53 gene product protein with a prolonged half-life that accumulates in the nucleus [11, 12].

This study identified p53 gene product accumulation in the neoplastic cells of partial, complete and invasive moles, but it was minimal in spontaneous abortions with hydropic changes. There are different reports about p53 immunoreactivity in abortuses with hydropic changes. In one study, p53 immunoreactivity was identified in 10% of trophoblastic cells of early gestation placentas (8-13 weeks). After 13 weeks, p53 protein was nearly undetectable [13]. In another study, p53 expression was detected in 9% of abortuses with hydropic changes [14]. However, in Bozom's study there was no case of single spontaneous abortion with hydropic changes that demonstrated staining with p53 protein [9].

The p53 immunoreactivity showed a difference between spontaneous abortions with hydropic changes and gestational trophoblastic disease in this study. However, the diagnostic value of p53 was low when used for gestational trophoblastic disease subgroups. Bozom [9] reported that p53 expression can be used to differentiate between molar and nonmolar pregnancies but negative p53 expression does not necessarily rule out molar pregnancy, particularly partial hydatidiform mole. Also, Cheung *et al.* [15] reported a significant difference in the levels of p53 ribonucleic acid between normal placentas and complete moles.

The increased overexpression of p53 in gestational trophoblastic disease seems to correlate with higher trophoblastic proliferation rates found mainly in complete and invasive hydatidiform moles. The p53 immunoreactivity was seen in primarily the germinative cell layer of the molar villi [10]. In this study, the p53 staining pattern was more extensive than the others, involving mainly the cytotrophoblastic cells in the invasive hydatidiform mole group. This observation supports the contention that p53 expression could be an indicator of proliferative activity [15] and may play a role in neoplastic transformation [9]. An additional immunohistochemical study performed by Persuad *et al.* [16] showed that mutation of the p53 tumor suppressor gene was seen primarily in choriocacarcinomas and trophoblastic elements of hydatidiform moles. On the other hand, Persuad *et al.* [16] also found that p53 overexpression was uncommon in partial hydatidiform moles and was minimal or absent in normal placentas.

In this study, the highest expressions of Ki-67 and PCNA were observed in invasive hydatidiform moles. This result suggests a more pronounced trophoblastic hyperplasia and proliferative activity in invasive moles. The results of PCNA expression in gestational trophoblastic diseases reported in the English literature show a high degree of variability because of different technical factors, such as storage, extent of fixation, or staining [5].

PCNA immunoreactivity in first-trimester molar placentas was significantly higher than that in normal placentas in Molykutty's study [17]. Whereas, Schammel *et al.* [18] reported that Ki-67, but not PCNA or p53 expression, identified differences between moles and hydropic nonmolar placentas. In a study on expression of proliferation markers in gestational trophoblastic disease, Kale [10] *et al.* noted that PCNA immunoreactivity was

minimal in normal placentas but more prevalent in invasive hydatidiform moles. In this study, Ki-67 and PCNA showed the highest expression in invasive hydatidiform moles, followed by complete hydatidiform moles and partial moles.

In spite of miscellaneous results [19] in the literature, in our study the extent of PCNA immunoreactivity was minimal in normal placentas and more extensive in hydatidiform moles, particularly in invasive hydatidiform moles. The more significant PCNA overexpression in invasive hydatidiform moles than in complete and partial moles indicates that the trophoblastic hyperplasia and proliferative activity of invasive hydatidiform moles is more evident than that of the others. This result is consistent with other previous studies [5].

The results of this study show that the evaluation of expressions of Ki-67, PCNA and p53 in cytotrophoblastic cells may be able to discriminate between spontaneous abortion with hydropic changes and gestational trophoblastic disease.

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