ORIGINAL ARTICLE

Diagnostic value of P63 in differentiating normal gestation from molar pregnancy

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Background: Considering the limitations of current pathologic methods in distinguishing two subtypes of hydatidiform mole and non-molar pregnancy, the utility of immunohistochemical markers in this regards and the importance of differentiating of mentioned pathologic patterns, in this study the expression of P63 in patients with complete hydatidiform mole (CHM), partial hydatidiform mole (PHM) and non-molar pregnancy was determined. **Materials and Methods:** In this study, formalin-fixed and paraffin-embedded tissues of 61 patients with definitive pathologic diagnosis of CHM, PHM and non-molar pregnancy retrieved. Diagnoses were based on the study of hematoxylin and eosin stained slides. Sections from all samples were stained for P63 marker using immunohistochemistry method. The nuclear immune reactivity of P63 marker in the three pathologic groups was determined by two pathologists. **Results:** P63 immune-staining was used to evaluate 20, 26 and 15 non-molar pregnancy, CHM and PHM cases, respectively. Mean ± SD of P63 nuclear immune-staining in molar pregnancy (CHM and PHM) and non-molar pregnancy were 32.4 ± 17.4 and 18.9 ± 17.2, respectively ($P = 0.006$). The means were significantly different between non-molar pregnancy and PHM ($P < 0.000$), CHM and PHM ($P = 0.02$) and non-molar pregnancy and CHM ($P = 0.04$). **Conclusion:** Considering the findings of the current study, though the nuclear immunoreactivity of P63 was higher in molar than non-molar pregnancy and in PHM than CHM, but using this marker alone is not suitable as a diagnostic test due to its low sensitivity and specificity. It could be used as adjuvant test in conflict cases. It is recommended to evaluate the role of other immunohistochemical markers like Ki-67 in this regard.

Key words: Complete hydatidiform mole, P63, partial hydatidiform mole, pregnancy, trophoblastic disease

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INTRODUCTION

Gestational trophoblastic disease (GTD) is a spectrum of 4 main clinicopathologic forms including, hydatidiform mole, invasive mole, placental site trophoblastic tumor and choriocarcinoma. The pathogenesis of these groups of disease is cellular proliferation of the placental villous trophoblasts.[1]

Hydatidiform mole characterized by the proliferation of different degrees of trophoblastic cells (both cytotrophoblast and syncytiotrophoblast) and vesicular swelling of placental villi and it results in abnormal pregnancy with an absent or an abnormal fetus. Based on morphologic and cytogenetic criteria, it classified in two syndromes as complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM).[2]

The incidence of hydatidiform mole is estimated to range from 0.57/1000 pregnancies to 2.0/1000 pregnancies, with a higher rate in Southeast Asia and Japan and lower rate in North America, Australia, New Zealand and Europe.[3,4]

Hydatidiform mole has a potential for malignant change. The risk of persistent gestational disease and mentioned potency is different in CHM and PHM and it is reported to be between 10-30% and 0.5-5% in CHM and PHM, respectively.[5] So, differentiation of these two subtypes of hydatidiform mole has an important management and prognostic implications.

Though there are well-described histopathologic criteria for distinction of CHM and PHM, but the definite diagnosis of the two pathologies is difficult due to variability in interobserver and intraobserver, specially during the early pregnancy.[6,7] In addition, distinction of hydropic abortion from the two subtypes of hydatidiform mole is another challenging issue.

Evidences indicated that some complementary methods to the pathologic interpretation such as genetic studies and immunohistochemistery could help us in this regard.[8,9]

Regarding genetic study, short tandem repeat polymorphism analysis has reported to be the best method. The method could not be used routinely

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because it considered expensive and time consuming method. It requires sophisticated equipment's and skilled personnel.[10] Immunohistochemistery has not mentioned limitations. In addition, it could be used in retrospective studies.[11]

Recent studies showed that impaired proliferation and specially apoptotic activity have a significant role in both pathogenesis and clinical outcome of hydatidiform moles.[12] P63 is one of the immunohistochemical markers in this field that its usefulness has been studied in many studies.[13-16]

P63, the p53 homolog, is a transcription factor localized on chromosome 3q27-29 and it is expressed in the cytotrophoblast cells of normal placenta and has a crucial role in the maintenance of stem cells and growth and development of different epithelial tissues.[17] Considering that P63 is not a classic tumor suppressor gene, there are controversies regarding the utility of this marker in differentiating CHM, PHM and non-molar pregnancy.[18]

Considering the limitations of current pathologic methods in distinguishing two subtypes of hydatidiform mole and non-molar pregnancy, the utility of immunohistochemical markers such as P63 in this regards and the importance of differentiating of mentioned pathologic patterns, the aim of this study was to determine the expression of P63 in patients with PHM, CHM and non-molar pregnancy.

MATERIALS AND METHODS

In this descriptive-analytic, cross-sectional study, formalin-fixed and paraffin-embedded tissues of 61 patients with definitive pathologic diagnosis of CHM, PHM and non-molar pregnancy retrieved from the pathology archives of Al-Zahra and Beheshti Hospital in Isfahan-Iran, from 2006 to 2011. All sections selected by the simple sampling method.

The Medical Ethics Committee of the Isfahan University of Medical Sciences approved the study protocol (research project number: 390206).

Diagnoses were based on the study of hematoxylin and eosin stained slides. The diagnoses were re-evaluated by two pathologists. PHM was diagnosed when the following four microscopic findings coexisted: Two populations of villi, enlarged villi with central cisterns, irregular villi showing scalloped borders and trophoblast pseudoinclusions and focal syncytiotrophoblast hyperplasia. CHM was diagnosed when a heterogeneous population of chorionic villi from small to extremely large villi with stromal edema and conspicuous cisterns were seen. These villi needed to show circumferential proliferation of both cytotrophoblastic and syncytiotrophoblastic cells with nuclear atypia. The presence of karyorrhexis in villous stroma further confirmed the diagnosis.[19]

Sections from all of the samples were stained for P63 marker using the immunohistochemistry method. The nuclear immunoreactivity of P63 marker in immunostained sections in three pathologic groups were determined by the same two pathologists.

Immunohistochemical staining

Multiple 3-μm thick sections of selected formalin-fixed, paraffin-embedded tissues were cut for immunohistochemical studies.

The sections were deparaffinised and rehydrated. Then, the sections were steamed in Tris EDTA buffer (pH = 9) in microwave for 15 min to facilitate antigen retrieval. H_2O_2 added to the samples for 10 min. Mouse monoclonal antibody (Biogenic Company – USA) of P63 was used for slides incubation at 4°C overnight. Immunohistochemical detection was performed using the polymer envision method. 3, 3 diaminobenzidine hydrogen peroxidase was used as chromogen. The sections were then counterstained with hematoxylin, dehydrated and mounted. Sections without antibody (using saline instead of antibody) and sections of cervical SCC served as negative and positive controls, respectively. The immunoreactivity of P63 in stained sections was examined by two pathologists. At least 100 trophoblastic cells were examined in each section. The percentage of the total number of nuclei counted in each section was determined. The mean of P63 nuclear immunoreactivity in each group was determined and compared with each other.

Statistical analysis

Obtained data analyzed using the SPSS version 18 for windows software.

One-way ANOVA test was used for comparing means of P63 nuclear immunoreactivity in the three studied groups. A Receiver operating characteristic curve was used to determine the cut-off point and the sensitivity and specificity of P63 in differentiating between groups.

RESULTS

In this study, 20.26 and 15 formalin-fixed, paraffin-embedded tissues and immunostained sections of non-molar pregnancy, CHM and PHM were studied, respectively [Figure 1]. Mean ± SD of P63 nuclear immunostaining in molar pregnancy (CHM and PHM) and non-molar pregnancy were 32.4 ± 17.4 and 18.9 ± 17.2, respectively (P = 0.006). Mean of P63 nuclear immunoreactivity in non-molar pregnancy, PHM and CHM is presented in Figure 2. The means were significantly different between non-molar

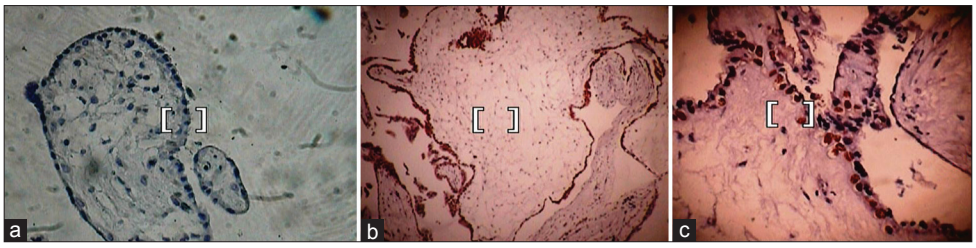

Figure 1: Immunohistochemical staining with P63 antibody in non-molar pregnancy (a), partial (b) and complete hydatidiform moles (c)

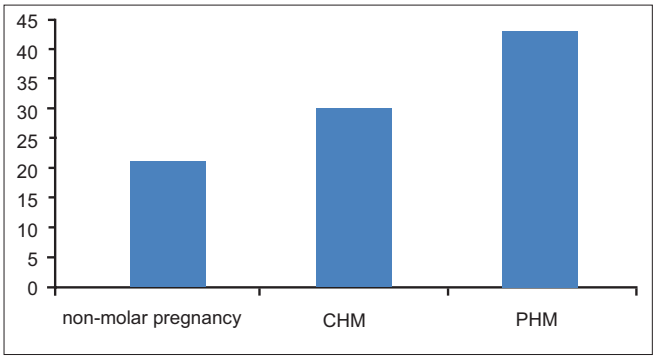

Figure 2: Mean of P63 immunoreactivity (mean of nuclei counted) in non-molar pregnancy, complete hydatidiform mole and partial hydatidiform mole

pregnancy and PHM ($P < 0.000$), CHM and PHM ($P = 0.02$) and non-molar pregnancy and CHM ($P = 0.04$).

ROC curves for determining cut-off point and sensitivity and specificity of P63 for differentiation of molar (CHM and PHM) and non-molar pregnancies are presented in Figure 3. The AUC was 0.735. The best cut-off point for differentiation of molar (CHM and PHM) and non-molar pregnancy was 17.5 with 75% sensitivity and 60% specificity. ROC curves for determining cut-off point and sensitivity and specificity of P63 for differentiation of CHM and PHM is presented in Figure 4. The AUC was 0.74. The best cut-off point for differentiation of CHM and PHM was 27.5 with 86% sensitivity and 58% specificity.

DISCUSSION

In this study, we investigated the utility of P63 in differentiating non-molar pregnancy, PHM and CHM. Means of P63 nuclear immune-reactivity was significantly higher in molar than non-molar pregnancy. P63 nuclear immune-reactivity was higher in PHM than CHM. Obtained sensitivity and specificity of P63 in distinguishing molar than non-molar pregnancy and PHM and CHM was not appropriate for using P63 marker as diagnostic test.

Several studies have investigated the role of P63 in distinguishing mentioned pathologies, but the results are controversial. Some reported higher expression of this marker in hydatidiform mole whereas others reported similar expression in molar and non-molar pregnancies.[13-16]

In a study in China, Chen *et al*. evaluated the expression of P63 in hydropic abortion and PHM. Expression of P63 was not significantly different between mentioned pathologies.[13]

In another study in China, Zhang *et al*. studied the expression of P63 in GTD and normal placenta. According to their results the expression was significantly higher in hydatidiform moles than normal placenta. In contrast to our results P63 expression was higher in CHM than PHM.[14]

The differences may be due to the laboratory methods. We used mouse 4A4 monoclonal antibody. They used 4A4 and anti-p40 antibodies. 4A4 detects total P63 isoforms whereas anti-P40 detects the pure DNp63 isoforms.

In a similar study in Iran-Mashhad, Erfanian *et al*. evaluated the usefulness of P63 marker in differentiating non-hydropic abortion, PHM and CHM. According to their findings P63 labeling index (positively stained nuclei/total number of nuclei counted) was significantly higher in molar than non-molar pregnancy. P63 labeling index was higher in CHM than PHM. They evaluate P63 in villous stromal cells, cytotrophoblasts and syncytiotrophoblasts separately and concluded that Ki-67 is better than P63 for differentiating studied pathologic features.[15]

Ramalho *et al*. in Brazil have investigated the utility of P63 Expression in hydropic abortion and GTDs. According to their results, P63 is useful to differentiate PHM and CHM from hydropic abortion and choriocarcinoma. They advised to use this marker in differentiate molar and non-molar pregnancies in challenging cases.[16] In contrast to our results, the P63 expression was not different in PHM and CHM.[16]

Regarding the higher expression of P63 in PHM than CHM, considering that some studies reported absence of P63 in choriocarcinomas due to having less differentiated cells and the higher tendency of CHM to malignancy than PHM, the finding could be explained. Further studies with larger sample size are recommended.

In this study, we determined the sensitivity and specificity of P63 in distinguishing molar from non-molar pregnancy

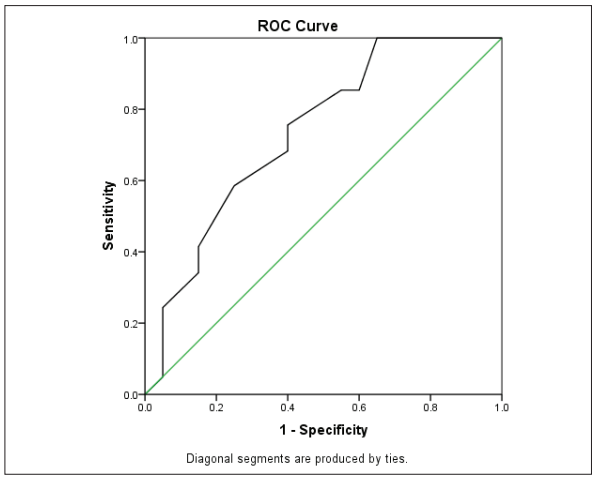

Figure 3: ROC curves for determining cut-off point and sensitivity and specificity of P63 for differentiation of molar (complete hydatidiform mole and partial hydatidiform mole) and non-molar pregnancies

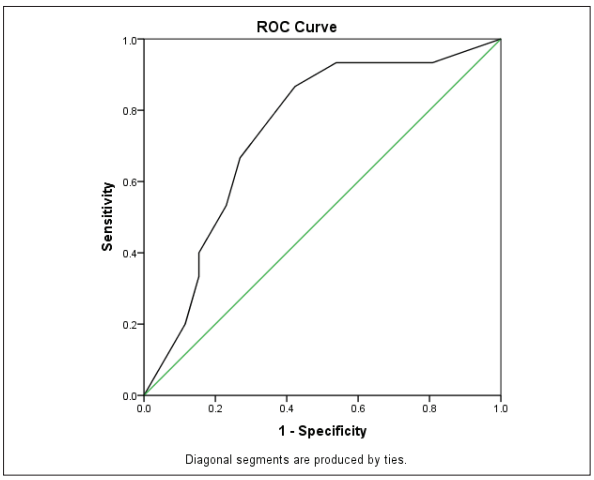

Figure 4: ROC curves for determining cut-off point and sensitivity and specificity of P63 for differentiation of complete hydatidiform mole and partial hydatidiform mole

and CHM from PHM. Though the sensitivity was more favorable in distinguishing CHM from PHM (86%) than molar from non-molar pregnancy (75%), but obtained results indicated that the sensitivity for both categories is not appropriate enough (<90%) for using this marker as diagnostic test separately. It seems that it could be used in conflicting cases as adjuvant method to conventional pathologic methods for distinguishing mentioned pathologies.

The limitation of current study was that we did not study the role of different isoforms of P63 due to not having enough facility. According to the previous studies, P63 encodes 6 isoforms. Three of them including DNp63a, DNp63b and DNp63 g who have anti-apoptotic activity and identified as oncogene. This group of isoforms is present in cytotrophoblastic cells. The reminder three isoforms including TAp63a, TAp63b and TAp63 g induce apoptosis owing to that it have the ability to trans activate the p53-related gene. The expression of this group of isoforms has been reported negative in all types of trophoblasts.[20,21] Evidences indicated that the limitation of immunohistochemistry is that it cannot distinguish DNp and Tap isoforms.[22] However, considering that TAp isoforms are almost negative in trophoblastic cells, so using immunohistochemistry for studied pathologies could be justified.

CONCLUSION

Considering the findings of the current study; though, the immunoreactivity of P63 was higher in molar than non-molar pregnancy and in PHM than CHM, but using this marker alone is not suitable as a diagnostic test owing to its low sensitivity and specificity. It could be used as adjuvant with current pathologic tests in conflict cases. It is recommended to evaluate the role of other immunohistochemical factors like Ki-67 in this regard.

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