

Differential Expression of p63 in Hydropic and Molar Gestations

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ABSTRACT

Objective: To observe the differential expression of p63 in hydropic and molar gestation.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate and Medical Centre, Karachi, from January 2006 to June 2013.

Methodology: Ninety placental biopsies including 30 cases each of hydropic abortions, partial hydatidiform mole and complete hydatidiform mole were analyzed for morphological features and results of immunohistochemical staining. Results were described as frequency. Significance was determined using test of proportions with significance at $p < 0.05$.

Results: Out of 30 cases of hydropic abortion, 6 were negative, 15 were weak, 4 were moderate and 5 showed strong degree of intensity for p63. Out of 30 cases of partial hydatidiform mole, 3 were negative, 2 showed weak, 4 showed moderate and 21 cases showed strong degree of intensity for p63. All 30 cases of complete hydatidiform mole strongly stained for p63.

Conclusion: The intensity of staining of p63 was stronger in cases of molar pregnancy as compared to hydropic abortion. There was loss of p63 expression in cytotrophoblastic cells in all abortions. In limited resources settings, where facilities for PCR/FISH and DNA ploidy analysis is not available, the authors advocate p63 in routine clinical practice to provide the most refined diagnosis of hydatidiform moles.

Key Words: Hydatidiform mole. Differential expression. p63. Trophoblastic hyperplasia.

INTRODUCTION

Gestational Trophoblastic Diseases (GTD) is a spectrum of cellular proliferations arising from placental villous trophoblasts.¹ The WHO classification of GTD includes Hydatidiform Mole (HM), Complete Hydatidiform Mole (CHM) and Partial Hydatidiform Mole (PHM), invasive mole, choriocarcinoma, placental site trophoblastic tumour and miscellaneous and unclassified trophoblastic lesions.²

Accurate diagnosis and classification of hydatidiform mole is important as the risk of persistent gestational trophoblastic disease including Choriocarcinoma (CC) is significantly high. The risk of CC in CHM is 10 - 30% and in PHM is 0.5 - 5%.³ Microscopic examination alone cannot always distinguish degenerative changes in non-molar placenta (hydropic abortion) from PHM and CHM.

A complementary method to the pathologic interpretation is Immunohistochemistry (IHC). Among the

immunohistochemical markers, p63 is of great value in studying the biologic behavior of gestational trophoblastic diseases; p63 is a member of the p53 gene family. The gene is located on chromosome 3q27 - 29. It encodes two primary transcripts, TAp63 and DNp63, which are controlled by two separate promoters, P1 and P2 respectively. TAp63 contains the Transactivation Domain (TAD), the DNA Binding Domain (DBD) and the Oligomerization Domain (OD). In contrast, DNp63 does not have any amino-terminal TAD. The DN terminal variants are generally regarded as dominant negative versions of p53 family members, as they can occupy promoter binding sites but fail to transactivate gene expression. p63 is required for limb and skin development.⁴ Indeed, the protein encoded by the p63 gene is highly expressed in the embryonic ectoderm.⁵ It is also involved in the modulation of gene expression associated with apoptosis, cell proliferation and inhibition of tumour progression in p53 dependent signaling pathways. Moreover, p63 can also regulate gene expression in p53 independent pathways for more specific genes that are associated with development, epithelial terminal differentiation and cell adhesion. Based on immunohistochemistry and RT-PCR, it appears that cytotrophoblast expresses the DeltaNp63 isoform.⁶

In Pakistan, very limited researches have been carried out to differentiate CHM and PHM from HA. The objective of this study was, therefore, to determine the differential expression of p63 in hydropic abortion and hydatidiform mole.

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METHODOLOGY

This cross-sectional study was conducted at the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate and Medical Centre, Karachi, from January 2006 to June 2013.

Over the 8 years study period, the authors came across 1000 placental biopsies including 200 Hydropic Abortion (HA) and gestational trophoblastic disease and 800 simple abortions. Amongst 200 cases, 87 were CHM, 62 were PHM, 7 were choriocarcinomas and 44 were HA. The researchers selected 90 placental samples including 30 cases of each i.e. HA, PHM and CHM for immunohistochemical staining. All clinically diagnosed cases suspected as gestational trophoblastic disease on the basis of clinical presentation and serum/urine HCG levels were included. Suboptimally-fixed tissue and inadequate material were excluded. Foreign nationals and Pakistanis living in foreign countries for more than 10 years were excluded from this study.

H&E stained slides were reviewed to confirm the diagnosis. The most representative section was used for immunohistochemical analysis. p63 (clone 4A4), mouse monoclonal antibody, procured from Santa Cruz was used in all immunohistochemical analysis. Antigen detection was done using HiDef detection HRP polymer system kit (ready to use) procured from Cell Marque. Skin was taken as internal positive control while PBS substituted primary antibody for negative control. Sections of approx. 3 µm were cut on to poly L-lysine coated slides and were deparaffinized and rehydrated. Antigen retrieval was achieved by microwave method using citrate buffer, slides were allowed to cool for 20 minutes and were then placed in UV block for 5 minutes. Tissues were covered with primary antibody at dilution 1:50 and were incubated for 1 hour at room temperature. Slides were then incubated first with Amplifier and then with HRP polymer for 10 minutes. Chromogen was applied for 20 minutes and all the slides were counter stained with hematoxylin, dehydrated and mounted. Between each step, the slides were washed with Phosphate Buffer Solution (PBS). The staining was quantitatively assessed as negative (no nuclear staining seen), 1+ (less than 10% nuclear staining is seen), 2+ (10 - 50% nuclear staining is seen) and 3+ (more than 50% nuclear staining is seen). The intensity of staining

was graded as no staining (0), weak staining (1+), moderate staining (2+) and strong staining (3+).

Data was collected in a well custom designed proforma and analyzed using Statistical Package for Social Sciences (SPSS) version 21. Fisher's exact test was applied where applicable. P-value less than 0.05 was considered statistically significant at 95% confidence interval.

RESULTS

Out of 1000 cases, 90 cases were selected for immunohistochemistry, including 30 each of HA, PHM and CHM.

Out of a total 1000 patients, who presented with abortions and GTD, the mean age was 27.86 ± 8.4 years (Table I). The age range of most of the patients of simple/ hydropic abortions was 21 - 30 years. In case of CHM, majority of the patients were belonging to the age group of 41-50 years while in PHM majority of the patients were from the age group of 21 - 30 years (Table II).

Results of immunostaining for p63 were analyzed on the basis of intensity of staining. Most of the cases of CHM (90%) showed highest degree of intensity (3+) and 10% showed 2+ intensity. In case of PHM, 70% showed 3+, 13.3% showed 2+, 6.6% showed 1+ and 10% were negative for p63. In case of HA, 16.7% showed 3+, 13.3% showed 2+, 50% showed 1+ and 20% were

Table II: Distribution of complete and partial hydatidiform mole in various age groups (n = 149).

Age groups (years)	Partial mole	Complete mole
10 - 20	9 (14.5%)	10 (11.5%)
21 - 30	30 (48.4%)	8 (9.2%)
31 - 40	7 (11.3%)	10 (11.5%)
41 - 50	16 (25.8%)	59 (67.8%)
Total	62 (100%)	87 (100%)

Chi-square=34.756; p < 0.001 * Significant.

Table III: Intensity of p63 immunoreactivity (n = 90).

Intensity of p63	Types of lesion			
	Hydropic abortion	Partial mole	Complete mole	Total
Negative	6 (20%)	3 (10%)	0 (0%)	9 (10%)
1+	15 (50%)	2 (6.6%)	0 (0%)	17 (18.9%)
2+	4 (13.3%)	4 (13.3%)	3 (10%)	11 (12.2%)
3+	5 (16.7%)	21 (70.0%)	27 (90.0%)	53 (58.9%)

Fisher's exact value = 44.23; p < 0.001 * Significant (between partial mole and complete mole).

Table I: Distribution of abortions and gestational trophoblastic diseases according to various age groups (n = 1000).

Age	Simple / hydropic abortions	Hydatidiform mole (CHM and PHM)	p-value	Invasive mole	Chorio-carcinomas	Placental site trophoblastic tumours
10 - 20	100 (11.8%)	19 (12.8%)	p < 0.001* Chi-square=300.106	-	-	-
21 - 30	544 (64.5%)	38 (25.5%)		-	7 (100%)	-
31 - 40	170 (20.1%)	17 (11.4%)		-	-	-
41 - 50	30 (3.6%)	75 (50.3%)		-	-	-
Total	844 (100%)	149 (100%)		-	-	7 (100%)

Overall mean age: 28.61±8.30 years, ranging from 13-50 years. Hydatidiform mole mean age: 42.85±3.71 years, ranging from 38-48 years.

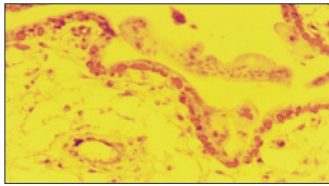


Figure 1: Photomicrograph: hydropic abortion negative for p63 IHC X 10 .

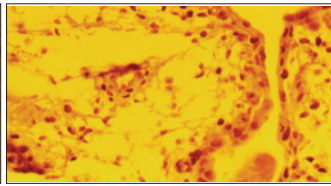


Figure 2: Photomicrograph: hydropic abortion 2+ positive for p63 IHC X 20.

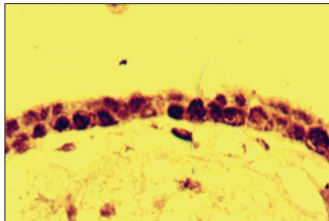


Figure 3: Photomicrograph: partial hydatidiform mole positive for p63 IHC X 40.

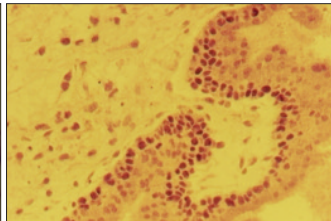


Figure 4: Photomicrograph: complete hydatidiform mole 3+ positive for p63 IHC X 10.

negative for p63. The results were found statistically significant and their p-value was less than 0.001 (Table III).

DISCUSSION

As histologic criteria may be insufficient to distinguish hydropic abortions from PHM and CHM, and pathologists may give a diagnosis reflecting uncertainty. Recently, an immunohistochemical staining technique has been reported as a good diagnostic adjunct complementary to histology.⁷ One of the advantages of this method is the ability to apply them retrospectively to sections of routinely formalin fixed and paraffin embedded tissue and there is no need for expensive or sophisticated equipments.⁸ Through the analysis of p63 expression in several normal tissues, Reis-Filho *et al.* verified that p63 is a marker of cytotrophoblastic cells in humans' placentas.⁹ Shih and Kurman classified the different trophoblastic subpopulations according to the expression of p63 isoforms.¹⁰ P63 encodes at least six major isoforms. Three isoforms (T) contain the transactivating domain (TA) and are able to transactivate the p53 related gene and induce apoptosis. The other three isoforms (N) lack the TA domain and have an antiapoptotic action, acting as oncogene.¹¹ According to literature, only N isoforms are present in cytotrophoblastic cells.¹²

In the present study, the utility of p63 in differentiating HA, PHM and CHM was investigated. Out of 30 cases of HA selected for immunohistochemistry, 6 cases were negative, 15 cases were weak, 4 cases were moderate and 5 cases showed strong degree of intensity for p63. Six cases that were negative, 15 cases that showed weak intensity and 4 cases that were moderate for p63 were confirmed to be hydropic abortions. Five cases that showed strong degree of intensity were morphologically re-evaluated and were finally diagnosed and labeled as

PHM. In contrast to these results, Zhang *et al.* reported that p63 expression was not significantly different between mentioned pathologies.¹³

A similar study in Mashhad, Iran, evaluated the usefulness of p63 marker in differentiating HA from PHM and CHM. It was concluded that p63 labelling index was significantly higher in molar than non-molar pregnancy.¹⁴

In this series, out of 30 cases of PHM selected for immunohistochemistry, 3 cases were negative, 2 case showed weak staining, 4 cases showed moderate and 21 cases showed strong degree of intensity for p63. Twenty one cases that showed strong staining were confirmed to be partial hydatidiform mole. Three cases with negative, 2 with weak and 4 cases with moderate degree of intensity were morphologically re-evaluated. Out of these 9 cases, 6 were confirmed to be partial hydatidiform mole and 3 were finally diagnosed as hydropic abortions.

Ramalho *et al.* reported that the intensity of immunostaining is stronger in PHM as compared to HA. According to them, p63 stains are well-differentiated cytotrophoblasts which is more abundant in PHM than HA. Furthermore, they advised to use this marker in differentiating molar and non-molar pregnancies in challenging cases.¹⁵

Chen *et al.* reported that there was no difference in positive rate and intensity of p63 immunostaining in HA and PHM.¹⁶ They observed that p63 expression is restricted in the cytotrophoblastic cells, not the intermediate trophoblasts and syncytial trophoblasts in HA and PHM. Therefore, p63 is a suitable marker for cytotrophoblastic cells.

In the present study, all of the 30 cases of CHM selected for immunohistochemistry were strongly stained for p63. These results correspond to those observed in studies by Zhang *et al.*¹³ and Heidarpour *et al.*¹⁷ where hydatidiform moles demonstrate significantly higher p63 indices than normal placentas.

CONCLUSION

The intensity of staining of p63 was strong in cases of molar pregnancy as compared to hydropic abortion. There was loss of p63 expression in cytotrophoblastic cells in all of abortions. In the limited-resource settings, the authors advocate p63 in routine clinical practice to provide the most refined diagnosis of hydatidiform moles.

Disclosure: This is a thesis based article.

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