ORIGINAL RESEARCH

The role of immunohistochemical markers in evaluating the malignant potential of molar pregnancies

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ABSTRACT

Background: Gestational trophoblastic disease are a group of benign and malignant entities, where the hydatidiform mole is the most common. p57 (KIP2) is the protein product of the paternally imprinted but maternally expressed gene. Because complete mole lack a maternal genomic component they are not expected to express imprinted genes that are normally expressed by the maternal allele and immunohistochemical analysis for p57(KIP2) has been shown to be a valuable tool in the diagnosis of a complete mole. p21 is a cell proliferation marker and the actively dividing cells show increased expression of p21 immunostaining. Based on this aim of our study is to evaluate the efficacy of immunohistochemical markers in differentiating partial hydatidiform mole from complete hydatidiform mole. Also to assess the role of immunohistochemical markers in assessing the malignant potential of molar pregnancies. **Methodology:** This is a retrospective and prospective study done at a tertiary care teaching hospital for a period of 2 years. A sample size of 52 cases are included in this study. All 52 cases were selected for this study and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry with a panel of 2 markers. Detailed history of the cases regarding age, symptoms, parity, radiological findings like ultrasonogram, history of previous pregnancy, details of gross characteristics were obtained. **Result:** 15 cases partial hydatidiform mole, 24 cases of complete hydatidiform mole, 11 cases of normal products of conception, 1 case of invasive mole and one case of choriocarcinoma were included in this study. The percentage of staining intensity of p57 is strong in 50%, weak in 28.8% and negative in 21.2%.In P21 -1+ staining was in 19 specimen, 2+ in 21 specimen and 3+ in 10 specimens. **Conclusion:** P57 is used as a potential marker in differentiating partial mole and hydropic abort us from complete mole, hence avoiding unnecessary DNA analysis. The over expression of p21 in complete mole, invasive mole and choriocarcinoma indicates its association with proliferative activity, hence it's a useful marker to assess the malignant potential of molar pregnancy. P21 targeted therapy is available in the market, so it can be used as a therapeutic marker.

Keywords: molar pregnancy, p21, p57, expression.

INTRODUCTION

Gestational trophoblastic disease are a group of benign and malignant entities, where the hydatidiform mole is the most common, and can be divided into complete hydatidiform mole and partial hydatidiform mole.[1] Hydatidiform mole is an abnormal placenta with villous hydrops and trophoblastic proliferation [2] Complete mole involves all the chorionic villi and has a diploid karyotype.[2] Partial mole is a hydatidiform mole with both normal villi and hydropic villi and focal trophoblastic proliferation. These lesions have triploid karyotype.[2] The distinction is made between complete and partial mole based on clinical, morphologic and genetic difference.[3] A complete mole is diploid, generally has a 46XX or a 46XY karyotype and is entirely androgentically derived. A partial mole usually contains an extra haploid set of paternally derived DNA and has 69 chromosomes.[4]

Histopathologically there is considerable interobserver and intraobserver variability in distinguishing hydropic abortion from hydatidiform mole and complete hydatidiform mole from partial hydatidiform mole. The distinction is important because approximately 10-30% of complete mole and 0.5-5% of partial moles can progress to persistent trophoblastic disease. In addition, distinction of hydatidiform mole from Non molar pregnancy can be

problematic in several situations, including: products of conception specimens (POCs) with abnormal villous morphology (a non-molar type of villous abnormality having some morphologic features suggestive of a PHM (partial hydatidiform mole) but lacking the diandric triploidy required for a definitive diagnosis of PHM, sometimes attributable to other genetic abnormalities such as trisomy; early non molar abortuses with prominent trophoblastic hyperplasia; hydropic abortuses; and mosaic/chimeric conceptions.[5]

However the histologic criteria are still questionable and histologic evaluation alone is prone to great interobserver variability. The risk of persistent disease and choriocarcinoma is more with complete mole. Persistent trophoblastic disease develops after a complete mole in 10% to 30% cases and after partial mole in 0.5% to 5% cases. Choriocarcinoma arises in 3% of complete mole and is rare with partial mole. Therefore distinguishing the molar pregnancy especially the more dangerous complete mole from its mimics during early gestation period is an important factor in management and prognosis. More challenges are experienced nowadays with advanced number of prenatal screening techniques that provide earlier clinical recognition and termination of abnormal pregnancies. p57 (KIP2) is the protein product of the paternally imprinted but maternally expressed gene, (CDKNIC) located on chromosome no 11p15.5. Because complete mole lack a maternal genomic component they are not expected to express imprinted genes that are normally expressed by the maternal allele and immunohistochemical analysis for p57(KIP2) has been shown to be a valuable tool in the diagnosis of a complete mole. [6]

Low or absent p57 (KIP2) expression likely plays an important role in the abnormal proliferation and differentiation of trophoblasts in tetraploid complete mole. In the villous tissue it is absent in synctiotrophoblast and strongly expressed in cytotrophoblast, villous mesenchyme and intervillous or intermediate trophoblast. Since complete mole lacks a maternal genome this gene is completely absent or weakly expressed. Partial mole, hydropic abortus show positive expression with p57 (KIP2) immunomarker. p57 IHC marker has an advantage of differentiating hydropic abortus from complete mole, a distinction not possible by ploidy analysis. Both complete mole and hydropic abortions on ploidy analysis show diploid karyotype which is well differentiated by p57immunohistochemistry [6]

Morphologic examination of products of conception (POC) forms the main diagnostic tool in the differential diagnosis of complete mole (CM) and partial mole (PM). However, the criteria are subjective and show considerable inter-observer variability. With early diagnosis and evacuation of molar pregnancies, the differentiation from early non-molar placentation, especially hydropic abortus (HA), is difficult. The p57KIP2 gene encoding for p57, a cell cycle inhibitor, is strongly paternally imprinted and expressed from the maternal allele. Because CM results from fertilization of an egg that has lost its chromosomes and the genetic material is completely paternally derived, p57KIP2 immunostaining is absent whereas HA and PM show positive staining. Thus, in equivocal cases, p57 immunohistochemistry (IHC) is an important adjunct to the diagnosis. Immunohistochemical study by using the markers p57 will aid in distinguishing hydropic abortion from hydatidiform mole and complete hydatidiform mole from partial hydatidiform mole. Also study of p21 will aid in assessing the malignant potential of molar pregnancy.

p21 is a cell proliferation marker and the actively dividing cells show increased expression of p21 immunostaining. This is the reason for its increased expression in first trimester compared to second trimester. PAK1 immune reactivity in cytotrophoblast and villous intermediate trophoblast demonstrated its proliferative capacity. Gestational trophoblastic diseases are characterized by abnormally high levels of trophoblast proliferation. Choriocarcinoma is a highly malignant tumor that may arise from the trophoblasts of any type of gestational event, and most commonly seen in the case of a complete mole. In choriocarcinoma, certain G1/S inhibitors, like p21 is over expressed.[7]

Based on this aim of our study is to analyze and review histopathologically proven cases of hydatidiform mole and hydropic abortions. Also to evaluate the efficacy of immunohistochemical markers in differentiating partial hydatidiform mole from complete hydatidiform mole. Also to assess the role of immunohistochemical markers in assessing the malignant potential of molar pregnancies.

MATERIALS AND METHODS

This is a retrospective and prospective study done at a tertiary care teaching hospital for a period of 2 years. Out of 5623 specimens received there were 412 specimens of products of conception. A sample size of 52 cases are included in this study. Out of which 15 cases of partial hydatidiform mole, 24 cases of complete hydatidiform mole, 11 cases of normal products of conception, 1 case of invasive mole and one case of choriocarcinoma are included in this study.

Detailed history of the cases regarding age, symptoms, parity, radiological findings like ultra-sonogram, history of previous pregnancy, details of gross characteristics were obtained from the patient files in the pathology registers. Freshly cut 4µ thick sections and Hematoxylin & Eosin stained sections of the paraffin tissue blocks of these specimens were reviewed.

All 52 cases were selected for this study and their representative formalin fixed paraffin embedded tissue samples were subjected to

immunohistochemistry with a panel of 2 markers. The results were recorded with photographs.

This study included patients with history of spontaneous abortions, patients who are diagnosed as a case of molar pregnancy by histopathological examination and patients willing to participate in the study are included. Whereas patients with other causes of abortions like ectopic pregnancy, infection, uterine abnormality were excluded.

All clinical data like age, parity and radiological findings like ultra-sonogram findings were the hematoxylin and eosin stained and mounted slides were obtained from the archives of pathology laboratory. All slides were reviewed and the paraffin embedded tissue blocks were retrieved from pathology lab and subjected to immunohistochemical studies.

Immunohistochemical analysis using p57 and p21 were done in paraffin embedded tissue samples using supersensitive polymer HRP system based on non-biotin polymeric technology. Sections with a thickness of 4 µ from selected formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The antigen is bound with mouse monoclonal antibody (DAKO) against p57 and p21 receptor and then detected by the addition of secondary antibody conjugated with horse radish peroxidase- polymer and Diaminobenzidine substrate.

RESULTS

The total number of cases received in our institute is 5623 over a period of 2 years,. There were 412 specimens of products of conception. A sample size of 52 cases are included in this study. Out of which 15 cases partial hydatidiform mole, 24 cases of complete hydatidiform mole, 11 cases of normal products of conception, 1 case of invasive mole and one case of choriocarcinoma were included in this study.

It was found that among the gestational trophoblastic diseases complete mole has the highest incidence of 46.2%. The second most common was partial molar pregnancy accounting for 28.8%. Choriocarcinoma and invasive mole has the least incidence of 1.9% each.

Age wise more common incidence was in 22-25 years age group (31%) followed by 26-29 years age group, above age 29 there is declining trend. There is increased incidence of GTD (3 case of complete mole, 5 products of conception) among the early gestational age up to 16 weeks accounting for about 58% compared to gestational age more than 16 wks. Complete mole has first and second trimester incidence of 25% and 75 % respectively where as partial mole has 100% incidence in second trimester.

Complete mole has increased incidence during early period of gestation before 16 week accounting for about 91.7% whereas partial hydatidiform mole has higher incidence after 16 weeks of gestation that is among 17 – 25 weeks accounting for about 16.7%. Abortion from non-molar gestation also has higher incidence up to 16wks of gestation accounting for about 66.7%.

Complete mole was common in age group of 22-25 years accounting for 46% and next higher incidence was in 18-21 years age group. Partial hydatidiform mole has higher incidence among 26-29 years accounting for about 60% and it has relatively less incidence among 18- 21 years. Non molar gestation has almost equal age distribution.

The incidence of GTD according to parity of patient, complete mole has highest percentage of incidence in primi 90% compared to partial mole which has only 10% incidence in primi. In this study partial mole has highest incidence among patients with parity 2. Bleeding PV followed by lower abdominal pain, hyperemesis are common presenting symptoms.

52 cases in our study 51% were clinically diagnosed as molar pregnancy and 39.2% cases were diagnosed as abortion. In our study 3 cases out of 24 cases of complete mole has previous history of molar pregnancy accounting for about 12.5 %, so complete mole has increased predilection to occur after previous molar pregnancy and it has previous bad obstetric history.

Table 1: P57 EXPRESSION IN GTD

Type Of GTD	P57			Total
	Negative	Strong	Weak	
Choriocarcinoma	1	0	0	1
Complete Hydatidiform Mole	22	2	0	24
Invasive Mole	0	0	1	1
Partial Hydatidiform Mole	0	14	1	15
Products Of Conception	0	9	2	11
Total	23	23	6	52

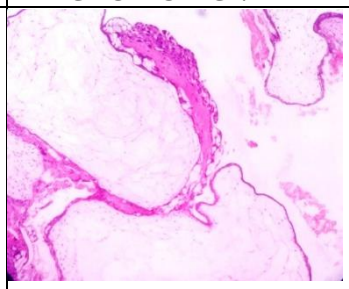
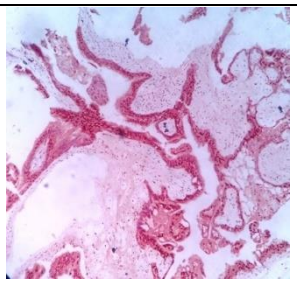
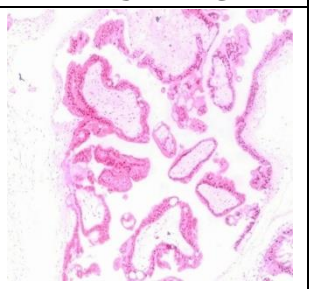
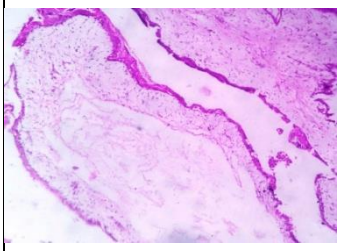
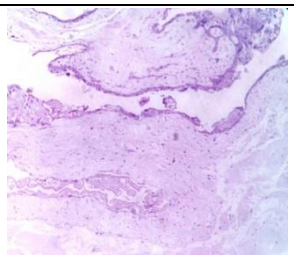
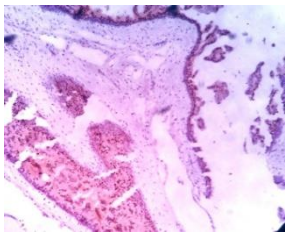
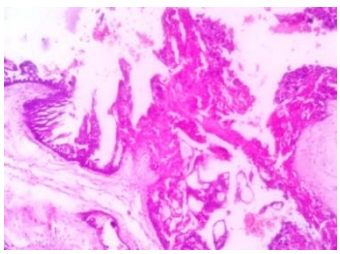
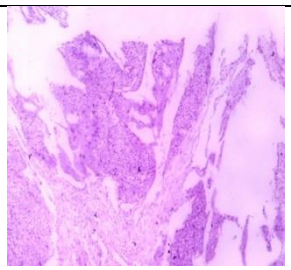
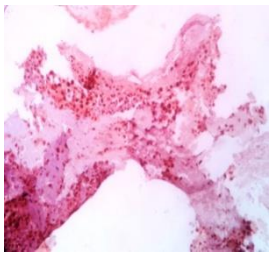
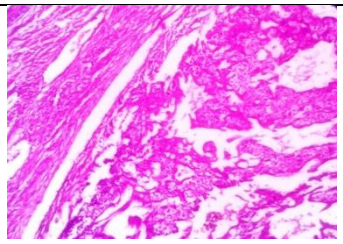
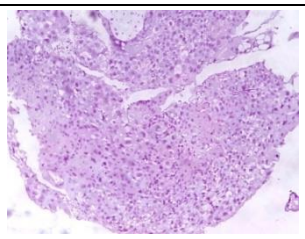
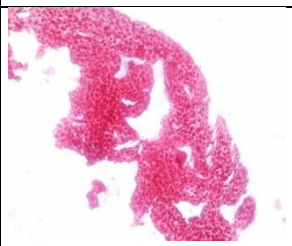
It is inferred that p57 expression was stronger in 14 out of 15 cases of partial mole accounting for about 93.3% and complete mole has predominantly negative expression of p57 in 22 out of 24 cases accounting for about 91.7% and weak staining intensity in 2 out of 24 cases accounting for about 8.3%. The invasive mole has weak staining intensity and choriocarcinoma stains negative with p57 immunohistochemistry. Non molar gestation has 81.8% strong positivity and 18.2 % weak staining intensity. The percentage of staining intensity of p57 is strong in 50%, weak in 28.8% and negative in 21.2%.

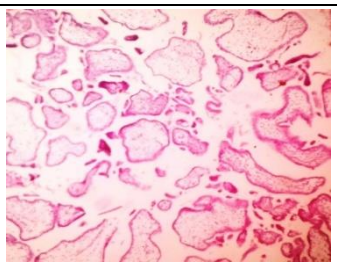
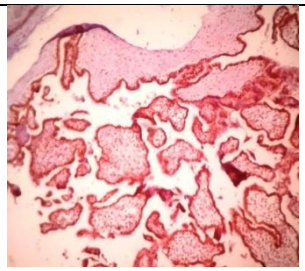
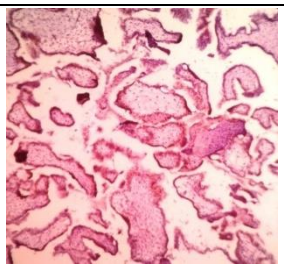
Table 2: P21 EXPRESSION IN GTD

Type Of GTD	p21				Total
	0	1	2	3	
Choriocarcinoma	0	0	0	1	1
Complete Hydatidiform Mole	1	3	12	8	24
Invasive Mole	0	0	0	1	1
Partial Hydatidiform Mole	1	11	3	0	15
Products Of Conception	0	5	6	0	11
Total	2	19	21	10	52

It has been inferred from our study that 8 cases of complete mole have 3 + staining intensity accounting for about 33% and 12 cases has 2 + staining pattern accounting for 50% .But partial mole has predominantly 1+ staining pattern accounting for 73%. Choriocarcinoma and invasive mole have 3+ staining intensity with p21.

Table 3: Histopathological features with p57 and p21 expression

GTD	CHORIONIC VILLI	P57 STAINING	P21 STAIING
PARTIAL MOLE			
COMPLETE MOLE			
INVASIVE MOLE			
CHORIO CARCINOMA			

PRODUCTS OF CONCEPTION			

It is also noted that p21 has 3+ staining intensity in 3 out of 19 cases and 2+ staining intensity in 15 out of 19 cases of gestational age up to 16 weeks accounting for about 15% and 78% respectively. In gestational age more than 16 weeks it has weak staining pattern of 1+ in 12 out of 14 cases accounting for about 85%.

DISCUSSION

In this study 41 cases of histopathologically proven gestational trophoblastic lesions and 11 cases of products of conception were reviewed and evaluated with IHC marker P57KIP2 expression and p21 expression to reevaluate the diagnosis and assess the neoplastic potential of the lesions.

The incidence of complete mole was found to be the highest in the sample, accounting for 46%. Next higher incidence was found for partial hydatidiform mole accounting for about 28%. The incidence of choriocarcinoma and invasive mole was very rare that is only one case was reported in our institute for past 5 years.

John R. Lurain, MD et al [8] Chicago stated in his journal of epidemiology, pathology, clinical presentation and diagnosis of hydatidiform mole that the 2 risk factors for the occurrence of molar pregnancy are extremes of maternal age and prior molar pregnancy.

In our study complete mole tends to occur in younger age group and 12.5 % of complete mole has previous history of molar pregnancy. Choriocarcinoma occurred in 29 years in our study and it has previous history abortion, it is significant because choriocarcinoma can occur following any gestational event. In our study invasive mole occurred at 50 years following the history of molar pregnancy 18 years back. In our study, based on gestational age, complete mole tends to occur during first trimester and early weeks of second trimester compared to partial mole which occurs relatively more common during second trimester of gestation.

Abnormal vaginal bleeding in early pregnancy is the most common presentation in molar pregnancy [9]. In our study also the common presenting complaints were bleeding per vaginum accounting for about 55%.

Due to marked interobserver and intraobserver variability in differentiating complete mole from partial mole and hydropic abortus, p57 marker was used. Secondly, hydatidiform mole is prone for persistent trophoblastic disease in the form of invasive mole or choriocarcinoma which develop in approximately 15-20% with complete mole and 1-5% with partial mole [10-15]. So, it is necessary to assess the malignant potential of molar pregnancy to prevent the occurrence of invasive mole and choriocarcinoma with the help of IHC marker p21.

52 cases comprising of 41 cases of histopathologically proven gestational trophoblastic lesions and 11 cases of products of conception The formalin fixed paraffin embedded sections were subjected to immunohistochemical analysis with p57 1nd p21.

EXPRESSION OF p57

Complete moles due to its lack of maternal genomic component, are not able to express the imprinted genes which are normally expressed by maternal allele results in negative expression for p57. Kihara et al. published the first report of perfect concordance between negative p57kip2 immunoreactivity and molar tissue of androgenetic origin. But partial mole which has maternal genome will express p57 in cytotrophoblast and villous stromal cells. However, immunohistochemical analysis for p57 cannot distinguish a PHM from hydropic abortion. [16]

In our study regarding the expression of p 57 in already diagnosed complete mole by H&E, 22 out of total 24 cases CHM were negative (92%) and remaining 2 cases showed strong staining intensity (8%) and serial sections were reviewed. Multiple sections studied in those two cases showed features of PHM and hence diagnosis was revised.

Regarding the expression of p57 IHC marker in partial moles 14 out of 15 cases shows strong staining intensity for p57(93.3%) and 1 out of 15 showed weak staining intensity (6.7%) which is statistically significant. Based on this expression of p57, it serves as a useful adjunct to differentiate between complete and partial mole. M. Paul et al. (2010) in USA [17] found that in his study all cases of normal product of conception showed strong positivity for p57KIP2 IHC marker.

In our study, P57 showed variable expression with 9 out of 11 cases showing strong positivity (81%) and 2 out of 11(19%) cases showed weak positivity. But based on histopathological feature we can differentiate between partial mole from normal placenta. In our Institute we had a rare case of partial mole with singleton live fetus. This has the most uncommon occurrence (0.005%-0.01%) of a singleton normal fetus with partial molar placenta. It has been

reported only seven times in extensively searched medical literature.

EXPRESSION OF p21

p21 is a cell proliferation marker and the actively dividing cells show increased expression of p21 immunostaining. This is the reason for its increased expression in first trimester compared to second trimester. PAK1 (p21 activated kinases) immunoreactivity in cytotrophoblast and villous intermediate trophoblast demonstrated its proliferative capacity.

Gestational trophoblastic diseases are characterized by abnormally high levels of trophoblast proliferation. Choriocarcinoma is a highly malignant tumor that may arise from the trophoblasts of any type of gestational event, and most commonly seen in the case of a complete mole. In choriocarcinoma, certain G1/S inhibitors, like p21 is over expressed.

In our study, 19 cases were of first trimester. Out of which 15 cases showed 2+ staining intensity accounting for about 93.8% and 3 cases showed 3+ staining pattern. But in second trimester p21 expression was decreased, 12 cases out of 14 shows 1+ staining intensity. Hence, most of first trimester pregnancy shows moderate expression of p21 and most of second trimester pregnancy sows weak expression of p21.

In case of molar pregnancy in our study, compete mole has increased expression of p21 compared to partial mole. Invasive mole and choriocarcinoma shows strong staining pattern with p21 indicating the highly malignant behavior of these GTD. In many other studies also it has been reported that p21 displays immunoreactivity the first-trimester cytotrophoblasts [18-20] and p21 shows an increased expression in gestational trophoblastic diseases.

CONCLUSION

P57 is used as a potential marker in differentiating partial mole and hydropic abort us from complete mole, hence avoiding unnecessary DNA analysis. The over expression of p21 in complete mole, invasive mole and choriocarcinoma indicates its association with proliferative activity, hence it's a useful marker to assess the malignant potential of molar pregnancy. P21 targeted therapy is available in the market, so it can be used as a therapeutic marker.

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