Diagnosis of molar pregnancy and persistent trophoblastic disease by flow cytometry

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SUMMARY Histopathological assessment and flow cytometric analyses were carried out on 32 placentas (representative of each trimester) and 88 molar pregnancies. Three first trimester placentas were triploid, and the remaining 29 placentas were diploid. Of the 88 cases originally diagnosed as molar pregnancies, 26 were triploid (two complete moles, 20 partial moles, and four hydropic abortions); 59 were diploid (46 complete moles, 10 partial moles, three hydropic abortions); one was tetraploid (partial mole); and two were DNA aneuploid (one partial mole, one complete mole). A significantly increased hyperdiploid fraction (a measure of cell proliferation) was detected in diploid complete moles ($p < 0.0001$) and cases of persistent trophoblastic disease ($p < 0.001$) when compared with diploid placentas and diploid partial moles. All seven cases of established persistent trophoblastic disease, for which follow up was available, were diploid and showed high hyperdiploid fractions within the range for diploid complete moles. These findings suggest that flow cytometric DNA measurements may be an important aid in the diagnosis of molar pregnancy. The high degree of cell proliferation found in this study may explain the premalignant potential of complete hydatidiform moles.

The distinction between partial moles and complete moles and their differentiation from hydropic abortions is a recurring and common problem for histopathologists.¹ The use of cytogenetic techniques,²⁻⁶ morphological studies,⁷⁻⁸ and enzyme markers³⁻⁴ have been of some assistance in making these distinctions. Complete moles are diploid, usually XX (rarely XY),⁹⁻¹² and are androgenetic in origin.² It has been suggested that complete moles with a 46XY karyotype may be more likely to progress to persistent trophoblastic disease¹² than complete moles with a 46XX karyotype. Partial moles are usually considered to be triploid (XXX or XXY), showing fetal presence and a maternal chromosomal contribution.⁵

The subdivision of molar and hydropic gestations has considerable prognostic importance. About 10% of patients with complete moles develop persistent trophoblastic disease,¹³ including the entities of invasive mole and choriocarcinoma, and a mole is the preceding gestation in at least half of all cases of choriocarcinoma.¹⁴ At present, the malignant potential of partial moles is uncertain.^{13 15 16} There are at least three published cases of persistent trophoblastic

disease¹⁷⁻¹⁹ and one case of fatal metastatic choriocarcinoma²⁰ developing after the diagnosis of a putative partial mole. It has been recommended that the clinical management of partial moles should be no different from that of complete moles,¹³ but this may result in unnecessary follow up of patients, which is both time consuming and expensive.

We have therefore investigated whether the identification of patients likely to progress to persistent trophoblastic disease could be assisted by flow cytometric analysis of DNA content.

Material and methods

CASES STUDIED

Sixty one placentas (representative of each trimester) and 114 molar pregnancies were retrieved from the files of the pathology departments of Leeds General Infirmary, Leeds, and the City Hospital, Nottingham.

PATHOLOGY

Routine 5 µm sections of all specimens, stained with haematoxylin and eosin, were reviewed independently by four histopathologists; a consensus diagnosis was used for comparison with flow cytometry. The placentas were subdivided into first, second, and third

trimester types, nearly all of which showed focal hydropic change on histological examination. The molar gestations were subdivided into complete moles and partial moles using previously accepted criteria.^{1 7 8 21 22}

Complete moles

Complete moles show pronounced hydropic change of all villi with central cistern formation. There is gross haphazard hyperplasia of both cytotrophoblast and syncytiotrophoblast with complete absence of fetal blood vessels and other features of fetal presence.

Partial moles

In partial moles hydropic change is focal with normal placental tissue in other areas. Blood vessels containing fetal red cells are often found together with other evidence of fetal presence. Trophoblastic hyperplasia is usually focal and mild. A further characteristic of partial moles are villi with irregular outlines and trophoblastic inclusions. A few partial moles may show a greater degree of trophoblastic hyperplasia, which is more characteristic of complete moles.¹³ Trophoblastic hyperplasia is an essential feature in differentiating partial moles from simple hydropic abortions.

Persistent trophoblastic disease

This condition results from persistence of trophoblastic tissue following a hydatidiform mole and includes the pathological entities of invasive mole and choriocarcinoma.

FLOW CYTOMETRY

Nuclear DNA measurements were performed using a modification of the method of Hedley *et al.*²³ Briefly, 50 μ m sections were cut from paraffin embedded material and transferred to glass slides. The sections were dewaxed in xylene and rehydrated by passing them through a series of alcohols (100%, 95%, 90%, 70%, and 50%). They were then washed twice in distilled water. The tissue was removed from the slide with a scalpel, placed in a test tube with 0.5% pepsin (Sigma Chemical Company, Poole) in 0.9% sodium chloride adjusted to pH 1.5 with 2N hydrogen chloride, and incubated at 37°C for 30 minutes in a water-bath. The cells were centrifuged at 2000 rpm, washed twice in distilled water, and stained by suspension in a solution (1 μ g/ml) of 4'-6'-diamidine-2-phenylindole dihydrochloride (Boehringer Mannheim, West Germany) in RPMI 1640 tissue culture medium at 20°C for 30 minutes. The cells were then syringed and filtered through four layers of muslin. Samples were analysed on a EPICS V flow cytometer (Coulter Electronics, Hialeah, Florida, USA). For excitation, a

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Fig 1 Two parameter analysis of DNA content v volume measured by Coulter volume accessory showing proliferating cells from tetraploid G0/G1 peak, which indicates polyploidy (arrow).

Coherent Innova-90 5W UV enhanced argon ion laser was used at 50 MW at a wavelength of 350 nm. A 408 nm interference filter removed scattered incident fluorescence.

Ten thousand nuclei were counted. DNA aneuploidy was defined as the presence of more than one G0/G1 peak.²⁴ The DNA index was calculated for DNA aneuploid samples, this being the ratio of the modal channel number of the abnormal G0/G1 peak to the modal channel number of the diploid G0/G1 peak.²⁴ Triploidy was defined as those cases with a DNA index of 1.4–1.6. Maternal tissue (decidua) for each case was used as an internal standard.

Definite evidence of polyploidy in the form of a small tetraploid population was present within the complete moles (fig 1). Because of this, cell cycle analysis was rendered invalid by standard cell cycle computer programs, and quantitation only of the number of cells with a DNA content greater than the 2c G0/G1 peak was performed. This fraction of cells, as a percentage, was termed the hyperdiploid fraction. Because of overlap of cell populations no attempt was made to measure the hyperdiploid fraction in the DNA triploid, DNA tetraploid, and DNA aneuploid samples. The relatively high mean half peak coefficient of variation (CV) of 9% in this series did not affect the ability to detect triploid populations. A high CV reduces the ability to detect minor ploidy abnormalities, but when dealing with two distinctly separate populations with major ploidy abnormalities, as in this study triploidy is detectable at CVs as high as 25%. A CV of 14% was used as the upper cut off level.

DEFINITIONS

Diploid, triploid, and polyploid tissue contain exact multiples of the haploid genome (n).

Diploid (2n)

Diploid tissue contains 46 chromosomes and would yield a single G0/G1 peak on a DNA histogram with a DNA index of 1.0.

Polyploidy (4n, 8n, and so on)

Polyploidy is a state where exact multiples of the normal diploid (n) genome are found. On a DNA histogram G0/G1 peaks would be seen at DNA indices of 1.0 and 2.0 if 2n and 4n populations were present and a further peak at 4.0 if an 8n population was found.

Triploid (3n)

Triploid tissue contains three sets of the haploid genome. In the presence of diploid maternal decidua and triploid molar tissue two peaks would be present at DNA indices of 1.0 and 1.5.

STATISTICAL ANALYSIS

Groups were compared using the Mann-Whitney U test and, where stated, the Kruskal Wallis test. Distribution of the hyperdiploid fraction for each group was expressed as the median value with the 5th and 95th centiles.

Results

PLACENTAS

Twenty nine of the 61 placentas studied were unsuitable for flow cytometric analysis, probably because of poor fixation. Three of the first trimester placentas ($n = 14$) were triploid; the other 11 diploid placentas

showed a median hyperdiploid fraction of 16.6% (5th–95th percentiles = 6.7%–20.8%) (fig 2). The median hyperdiploid fractions for the second ($n = 10$) and third ($n = 8$) trimester placentas were 11.8% (5th–95th percentiles = 6.7%–20.7%) and 14.2% (5th–95th percentiles = 13.0%–19.0%), respectively. The overall median hyperdiploid fraction for all diploid placentas was 14.9% (5th–95th percentiles = 6.8%–22.4%) (table 1). No significant difference in the hyperdiploid fraction was found between the first, second, and third trimester placentas (Kruskal Wallis test).

MOLAR GESTATIONS

Ploidy status

Twenty six of the 114 molar gestations were unsuitable for flow cytometric analysis. Of the remaining 88, 59 were diploid, 26 triploid, one tetraploid, and two DNA aneuploid with DNA indices of 1.22 and 2.3, respectively (table 2).

Table 1 Median percentage hyperdiploid fractions for placentas

	No	Median % hyperdiploid fraction (5–95th percentiles)
First trimester	14*	16.6 (6.7–20.8)
Second trimester	10	11.8 (6.7–20.7)
Third trimester	8	14.2 (13.0–19.7)
Overall	32	14.9 (6.8–22.4)

*Includes three triploid placentas which were excluded for the calculation of the hyperdiploid fraction in the first trimester placentas.

Fig 2 DNA histograms: (a) diploid placenta; (b) diploid partial mole.

Table 2 Ploidy status and histological diagnosis of molar pregnancies

Ploidy status		Histological diagnosis		
		Complete mole	Partial mole	Hydropic abortion
Diploid	59	46	10	3
Triploid	26	2	20	4
Tetraploid	1	—	1	—
Aneuploid	2	1	1	—
Total	88	49	32	7

Histopathological diagnosis

Forty six complete moles, 10 partial moles, and three hydropic abortions were diploid. Twenty partial moles, two complete moles, and four hydropic abortions were triploid. The one tetraploid molar gestation was considered to represent a partial mole. The two molar gestations showing DNA aneuploidy were classified as a complete mole and a partial mole.

Diploid molar gestations

Analyses of the 59 diploid molar gestations proved interesting. The 46 diploid molar gestations classified as complete moles (fig 3) showed a median hyperdiploid fraction of 42.2% (5th–95th percentiles = 23.3%–56.2%), which is well outside the range for placentas ($p < 0.0001$). Included with these 46 cases were seven clinically confirmed cases of persistent trophoblastic disease, including invasive mole. The median hyperdiploid fraction for these was 38.8% (5th–95th percentiles = 30.2%–51.5%), which is within the range for diploid complete moles (NS) and outside that for placentas ($p < 0.0001$). The 10

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diploid molar gestations classified as partial moles (fig 2) showed a median hyperdiploid fraction of 16.8% (5th–95th percentiles = 10.1%–26.6%), which is almost completely within the range of placentas but significantly different from that for diploid complete moles ($p < 0.0001$) and the seven cases of persistent trophoblastic disease ($p < 0.001$) (fig 4). Three of the diploid molar gestations were reclassified as hydropic abortions and showed hyperdiploid fractions of 20.1%, 18.0%, and 8.8%, falling within the range for placentas.

Triploid molar gestations

None of the 26 triploid molar gestations (fig 3) included examples of persistent trophoblastic disease.

Discussion

For histopathologists the distinction between complete moles and partial moles and their differentiation from hydropic abortions is not easy,¹ especially when there is no access to cytogenetic techniques or the tissue is unsuitable for chromosomal analysis. We have found that the present histological and morphological criteria are not as reliable in practice as has been suggested by other workers.^{8,25} Assessment of the degree of villous hydropic swelling and trophoblastic hyperplasia can be highly subjective. Central cyst formation within villi can be seen in both partial moles and complete moles along with trophoblastic "inclusions" and scalloping of villi.^{7,8} Probably the most useful histological criterion for differentiating partial moles from complete moles (in the absence of cytogenetic techniques) is the presence of fetal parts, including fetal red blood cells within the villous vasculature of partial moles.⁸ It could be argued even in

Fig 3 DNA histograms: (a) triploid partial mole; (b) diploid complete mole.

Fig 4 Median percentage hyperdiploid fractions (5th-95th percentiles) for all diploid placentas and molar pregnancies. Significant differences were shown between placentas and diploid complete moles ($p < 0.0001$) and cases of persistent trophoblastic disease ($p < 0.001$). No significant difference was shown between placentas and diploid partial moles or between diploid complete moles and cases of persistent trophoblastic disease.

these cases that a twin pregnancy with a complete mole cannot be excluded. It is therefore important that in normal clinical practice the diagnosis of the molar pregnancy should not be made on gross and histological features alone and should take account of the clinical findings (including ultrasound examination and β -human chorionic gonadotrophin estimations) when available.¹

We found that diploid complete moles exhibited hyperdiploid fractions well outside the normal range for diploid placentas ($p > 0.0001$) (fig 4). Among these 46 cases were seven cases of clinically confirmed persistent trophoblastic disease. These were all diploid and showed hyperdiploid fractions within the range for complete moles. Increased cell turnover is found in a variety of potentially neoplastic states—for example, ulcerative colitis.²⁶ The increased cell proliferation (termed hyperdiploid fraction) seen in complete moles compared with that seen in normal placentas may explain the neoplastic potential of complete moles.

Statements that partial moles have no malignant sequelae have been strongly contradicted by others,^{13 15 16} with three published cases of persistent trophoblastic disease¹⁷⁻¹⁹ and one case of fatal metastatic choriocarcinoma.²⁰ In only one of these cases had chromosomal studies been carried out. We found that all our clinically confirmed cases of persistent trophoblastic disease were diploid with hyperdiploid fractions characteristic of diploid complete moles (fig 4). None of the partial moles in our series have yet progressed to persistent trophoblastic disease during follow up.

Partial moles are usually considered to be triploid with evidence of fetal presence and a chromosomal contribution from the female gamete.⁵ Not all triploid abortions are partial moles,^{27 28} as confirmed in our series. We believe we may have isolated a third type of molar gestation, the diploid partial mole with histopathological features of a partial mole, a diploid DNA content, and a low hyperdiploid fraction more characteristic of normal placentas and hydropic abortions. These 10 cases may represent the 46XX partial moles described by Szulman and Surti^{7 8} and the three cases of partial mole with diploid karyotype described by Teng and Ballon.²⁹ In the three cases described by Teng and Ballon the partial mole occurred in conjunction with a live fetus and normal placental tissue; the possibility of twin pregnancy could not be excluded. The same workers went on to suggest that the diploid partial mole is a distinct entity with malignant potential since in each of their three cases β -human chorionic gonadotrophin concentrations were increased, with two patients requiring chemotherapy. Further studies are clearly required to assess the biological behaviour of diploid partial moles.

In addition to the group of diploid partial moles flow cytometry showed one case of partial mole with a DNA tetraploid DNA content and one case each of complete mole and partial mole showing DNA aneuploidy. Occasional cases of tetraploidy have been described in both complete^{4 30} and partial moles.³¹ The importance of DNA aneuploidy in molar pregnancies has not yet been established, although it is a recognised occurrence.⁵

Flow cytometric analysis of two of the molar gestations histologically classified as complete mole showed them to be triploid. It is possible that inadequate sampling of the molar tissue resulted in histopathological misdiagnosis. The possible entity of a triploid complete mole of androgenetic origin cannot be completely excluded, however, until further studies have been performed.

In conclusion, we believe that flow cytometric analysis of DNA content has certain advantages over current cytogenetic techniques in that analyses can be

performed rapidly on formalin fixed, paraffin embedded tissues²³ either retrospectively or at the time of receipt. The versatility of the technique is emphasised by the clear definition of the different groups obtained even with the relatively high mean CV of 9% in this series. The results of this study show that the flow cytometric measurements of DNA content may be an important aid in the accurate diagnosis of molar gestations since the ploidy status for a given specimen can be rapidly determined along with the hyperdiploid fraction in diploid cases. The use of the flow cytometer in the identification of those patients at risk of developing persistent trophoblastic disease needs to be studied further. In our series the seven cases of clinically confirmed persistent trophoblastic disease were diploid and showed hyperdiploid fractions characteristic of a complete diploid mole. As a research tool flow cytometric measurement of DNA content should provide further insight into the origins and, more importantly, the risk of persistent trophoblastic disease for each subtype of hydatidiform mole.

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