Original paper

Molecular and histological characteristics of early triploid and partial molar pregnancies

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Molar pregnancy has the highest incidence of all gestational trophoblastic diseases. This is a heterogeneous group of diseases, composed of precancerous lesions and gestational trophoblastic tumours. The hydatidiform mole is characterised by varying degrees of proliferation of syncytiotrophoblastic and cytotrophoblastic cells and stromal oedema. Based on established morphological and cytogenetic criteria, molar pregnancy is divided into partial and complete. The risk of persistent trophoblastic disease is higher in complete moles compared with partial moles.
The aim of this study was to assess the importance of additional molecular methods as a conjunction to the standard histopathological analysis to accurately determine the type and origin of triploidy and to detect partial molar pregnancy.
This study examined a total of 24 cases of triploidy. Apart from the detailed histomorphological analysis, a molecular analysis of the placental tissue and maternal DNA was also performed.
Digynic triploidy was found in 15 cases, whereas diandric triploidy was found in nine of the cases. The results showed that due to the histomorphological overlap between partial molar pregnancy and hydropic abortions, concomitant histopathological analysis of the placental tissue and molecular analysis of the placental and maternal DNA can lead to correct diagnosis.

Key words: partial mole, spontaneous abortion, triploidy.

Introduction

Gestational trophoblastic disease is a heterogeneous group of diseases that includes precancerous lesions such as hydatidiform mole and lesions with malignant potential, grouped together in the category of gestational trophoblastic tumours (invasive mole, choriocarcinoma, placental site trophoblastic tumour, and epithelioid trophoblastic tumour). The incidence of the precancerous lesions or molar pregnancy is much higher than the incidence of other gestational trophoblastic tumours [1, 2, 3]. It is estimated at 0.57-2/1000 pregnancies. Higher incidence occurs in Southeast Asia and Japan and lower in Europe, North America, Australia, and New Zealand [4]. The risk of persistent trophoblastic disease is higher in complete hydatidiform mole, compared with partial mole [1, 5]. For example, invasive hydatidiform mole occurs in 0.5-5% of cases of partial molar pregnancy, whereas the risk of subsequent development of choriocarcinoma is only 0.5% [6].

Based on well-established morphological and cytogenetic criteria, molar pregnancy is divided into partial and complete [4].

Morphological diagnostic criteria for the determination of the type of pregnancy (molar or non-molar) are clearly defined in the literature. For example, the microscopic diagnostic characteristics of partial molar pregnancy are also clearly defined, such as: presence of two populations of chorionic villi (enlarged, hydropic, and normal sized villi with fibrotic stroma); absent or minimal trophoblastic hyperplasia; presence of enlarged cavitated villi or villi with scalloped borders often containing oval trophoblastic pseudoinclusions; and presence of foetal red blood cells in the vasculature [7]. However, especially in early pregnancy, the differences between classical pathological changes in hydropic abortion and various types of molar pregnancies are subtle. Thus, when molecular methods were used, considerable variation in the interpretation of these morphological criteria was observed [1, 2, 3, 8, 9].

According to the European Society of Human Reproduction and Embryology, early spontaneous abortion is defined as clinical miscarriage, which occurs before the twelfth week of gestation. The incidence of early spontaneous abortions is about 15%, with considerable variation depending on maternal age [10].

The aim of the study was to assess the importance of additional molecular methods in conjunction with detailed histomorphological analysis to accurately determine the presence and origin of triploidy, and to accurately diagnose partial molar pregnancy.

Material and methods

For this study, we selected a group of 24 consecutive cases of triploidy from a total group of 231 analysed cases of early spontaneous abortions. Apart from the standard histopathological analysis, all 231 cases were further analysed using molecular methods. All cases showing triploidy were selected for further analysis in this study. In order to ensure maximal objectivity, all 24 cases were also tested with the p57 immunohistochemical marker, and all showed positivity in the villous cytotrophoblasts and stromal cells.

The curettage material was first washed from residual blood and then immersed in water. Using the difference in their appearance and floating characteristics, representative fragments of placental and decidual tissue were selected by an experienced pathologist and were frozen in liquid nitrogen. A separate set of placental and decidual tissue fragments was fixed in formalin and then routinely processed and paraffin embedded for microscopic morphological analysis. A blood sample from the mother was also obtained. For the histopathological diagnosis of partial molar pregnancy, four major criteria were established: 1) presence of two populations of chorionic villi; 2) enlarged oedematous villi with central cavitations; 3) irregular, jagged contours of the villi with trophoblastic pseudoinclusions; and 4) focal hyperplasia of the syncytiotrophoblasts; whereas presence of foetal erythrocytes was used as a fifth optional criterion.

For each of the samples, a detailed microscopic examination of haematoxylin-eosin stained tissue sections was performed by a single pathologist experienced in gynaecologic pathology, without knowledge of the results from the molecular analysis. Cases were scored as partial moles when all four previously described major criteria were present. The presence or absence of foetal erythrocytes was also evaluated.

A molecular analysis was performed in order to detect the possible chromosomal abnormality in the placental tissue and to reveal the origin of the extra chromosomal set. We used the quantitative fluorescent polymerase chain reaction method (QF-PCR). The analysis was performed on an 3100 Genetic Analyser and an ABI-PRISM 3500 Genetic Analyser. Using multiplex PCR reaction, four DNA markers on chromosomes 18 (D18S535, D18S391, D18S390, and D18S386), 21 (D21S1435, D21S1446, D21S1411, and D21S1414), and 13 (D13S631, D13S305, D13S258, and D13S1817) and two markers on the X chromosome (DXS6803 and HPRT) and amelogenin locus (AMXS) were analysed.

Results

The molecular analysis of the maternal blood or decidual tissue showed normal female genotype 46, XX in all 24 cases. These results were further used to assess the parental origin of the alleles in the placental tissue.

The results of the molecular analysis of the placental tissue showed that triploidy was found in 10% (24/231) of the cases. Of the 24 triploids further analysed, the extra chromosomal set was of paternal origin in nine (37.5%) cases, and of maternal origin in 15 cases (62.5%). Three different genotypes were present and their distribution in correlation with the origin of the triploidy is given in Table I.

According to the displayed distribution, the genotype 69, XXY more often had maternal origin of the extra chromosomal set (10 vs. 5), as well as the

Table I. Distribution of foetal genotypes in comparison to the origin of the triploidy

Genotype n (%)	Paternal n = 9	Maternal n = 15	p value
69, XXY	5 (55.56)	10 (66.67)	0.22
69, XXX	2 (22.22)	5 (33.33)	
69, XYY	2 (22.22)	0	

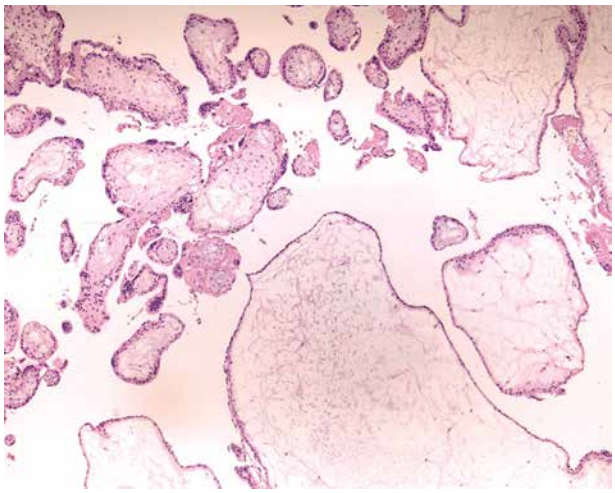

Fig. 1. Presence of two types of chorionic villi (haematoxylin and eosin, 40×)

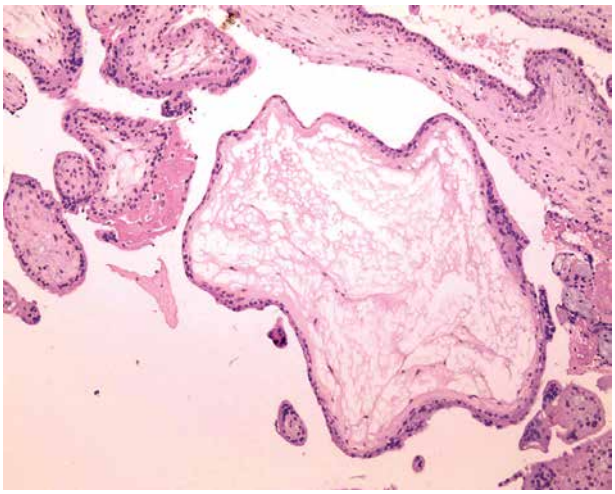

Fig. 2. Pronounced villous stromal oedema (haematoxylin and eosin, 40×)

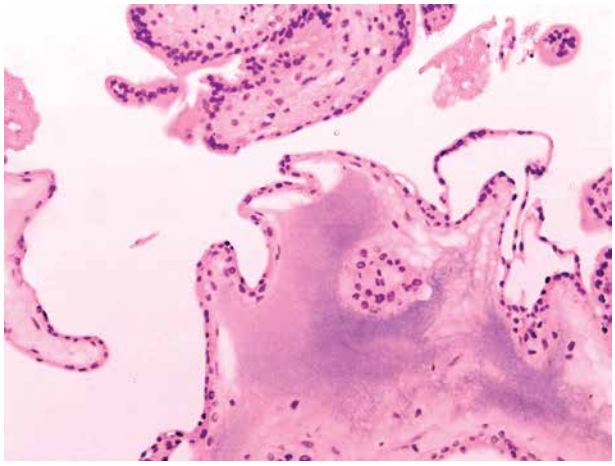

Fig. 3. Presence of trophoblastic stromal inclusions (haematoxylin and eosin, 100×)

genotype 69, XXX (5 vs. 2). The difference was not statistically significant.

When each of the five defined microscopic criteria suggestive of partial molar pregnancy (Fig. 1-3) was individually analysed (Table II), only the distribution of the presence of two types of chorionic villi had statistical significance. Thus, two populations of chorionic villi were significantly more common in diandric triploidies ($p = 0.00075$).

Trophoblastic pseudoinclusions were also more often detected in diandric triploidies – 66.67% (6/9) vs. 26.67% (4/15) digynic triploidies, but this difference was not statistically significant ($p = 0.09$).

Therefore, we further assessed the distribution of triploids of maternal or paternal origin depending on the total number of positive findings for the five morphological criteria of partial molar pregnancy: presence of two populations of chorionic villi, irregular villous contours, trophoblastic pseudoinclusions, focal trophoblastic hyperplasia, and the presence of foetal erythrocytes (Table III). In one diandric triploidy and two digynic triploidies, none of the morphologic criteria were found, while all five analysed criteria were detected in five of the nine diandric triploidies, but only in one digynic triploidy.

The descriptive statistics of the number of predictors was performed with a median number of predictor factors of five (range 3-5) in the diandric triploidies, and a median of two (range 2-4) in the digynic triploidies. In fact, half of the diandric triploidies had a positive finding for all five morphological criteria, whereas half of the digynic triploidies had more than two morphological criteria. The statistical analysis confirmed the difference in median number of predictor factors between the two groups to be statistically significant ($p = 0.04$), suggesting that diandric triploidies had a significantly greater number of diagnostic parameters suggestive of partial hydatidiform mole.

Discussion

Partial molar pregnancy is usually triploid and contains an additional haploid set of paternal chromosomes, giving a total of 69 chromosomes. As given in the literature, in about 90% of the cases the paternal triploidy is derived from dispermic fertilisation of a haploid egg (androgenetic triploidy). Less often (10%) it is a consequence of fertilisation of a haploid egg with unreduced diploid sperm (diandric triploidy) [5, 11].

Although diandry was once thought to account for > 80% of triploids, more recent studies demonstrate that digyny is responsible for more cases of triploidy than previously thought. Digynic triploidy may be the result of fertilisation of a diploid ovum by a single sperm, with the diploid ovum being the result of an error in either the first (MI) or second (MII) meiotic division [11]. Whatever the origin of the extra chro-

Table II. Histomorphological characteristics in paternal (diandric) and maternal (digynic) triploidies

	Paternal origin (n = 9)	Maternal origin (n = 15)	p value
Two types of villi			
present (n = 16)	2 (12.50%)	14 (87.5%)	0.00075
absent (n = 8)	7 (78.5%)	1 (12.5%)	
Irregular villous contours			
absent	1 (12.11%)	7 (46.67%)	0.18
present	8 (88.89%)	8 (53.33%)	
Trophoblastic pseudoinclusions			
absent	3 (33.33%)	11 (73.33%)	0.09
present	6 (66.67%)	4 (26.67%)	
Focal trophoblastic hyperplasia			
absent	1 (11.11%)	7 (46.67%)	0.18
present	8 (88.89%)	8 (53.33%)	
Foetal erythrocytes			
absent	4 (44.44%)	3 (20%)	0.36
present	5 (55.56%)	12 (80%)	

mosomal set, according to McFadden *et al.* (2002), who analysed 14 cases of triploidy, virtually all triploids are a consequence of an error in the ovum: either a division error leading to digyny or an abnormality of the ovum that fails to block fertilisation by more than one sperm [11, 12].

However, in our study, 62.5% were digynic triploidies. This incidence of the maternal origin of the extra chromosomal set was higher than expected, and may be due to the selection method used. Namely, our cases are in fact consecutive cases of triploidy detected in early spontaneous abortions irrespective of the histological characteristics. If only samples with morphological characteristics of partial molar pregnancy were analysed, or late spontaneous abortion cases were included, probably the percentage of diandric triploidies would be higher. For example, Zaragoza *et al*. (2000) in their series of 91 cases, found 69% of the triploids to be diandric or of paternal origin. However, their heterogeneous group of cases also included late spontaneous abortions and cases with advanced maternal age or placental morphologic abnormality [13]. In that particular study, the majority of diandric triploids had developmental ages in excess of 8.5 weeks, whereas most of the digynic triploids have aborted earlier, before developmental age 8.5 weeks, and this difference in distribution was highly significant. Other authors also found a predominance of paternal triploidy over maternal, but the cases examined were in the 11-14th week of pregnancy [14]. On the other hand, McFadden *et al*. (2006) reported higher incidence of digynic triploidy, but their series, the same as ours, evaluated early spontaneous abortions [15]. Since only early spontaneous abortions up to the 11th week of gestation were included in our study, this could partially explain the predominance of digynic triploidies.

Table III. Distribution of triploids of maternal or paternal origin depending on the total number of positive findings for the five morphological criteria of partial molar pregnancy

	Paternal origin (n = 9)	Maternal origin (n = 15)	p value
Number of predictors			
None	1 (11.11)	2 (13.33)	0.
1	0	5 (33.33)	
2	0	1 (6.67)	
3	3 (33.33)	3 (20)	
4	0	3 (20)	
5	5 (55.56)	1 (6.67)	
Median (IQR)	5 (range 3-5)	2 (range 1-4)	0.0397

The overall detection rate of triploidy in this study was 10%. According to the literature, triploidy can be detected in 10-18% of abortion specimens [16, 17].

We did not find a significant difference in the distribution of the different genotypes (69, XXX and 69, XXY) in correlation with the origin of the triploidy. However, the genotype 69, XXY was found to be the predominant genotype in both groups. Similar results were published in the literature from other authors who found similar genotype distributions between the paternal and maternal triploidies, with the genotype 69, XXY being the predominant genotype [18].

Although the histopathological or morphological criteria for the diagnosis of molar pregnancy are clearly defined in the literature, unfortunately they are not always present in histological slides, especially in cases of early partial molar pregnancy [19, 20, 21, 22, 23].

The association of the partial hydatidiform mole with diandric triploidy was originally suggested by cytogenetic studies [24] and by molecular analyses of partial moles [25]. In the present study, some of the histological characteristics of partial molar pregnancy were more common in diandric triploidy (5/9) than in digynic triploidy (1/15). Unfortunately, it is still unclear whether all triploids of paternal origin are partial hydatidiform moles. In fact, in our study there were several cases that did not show any of the morphological signs of partial molar pregnancy. A reason for this finding could be the early gestational age.

Some authors, such as Zaragoza *et al*. (2000), speculate that maybe the characteristic features of partial moles might not be evident until relatively late in gestation, leading to misdiagnosis of early aborting triploids as nonmolar pregnancies [13]. They found a strong relationship between age and partial moles, with molar diagnoses being assigned to 0%, 42%, 56%, and 78% of diandric triploidies of developmental ages < 6, 6–8.5, 8.5–11.5, and > 11.5 weeks, respectively [13]. In our study, all four major criteria for partial mole were found in 56% of diandric triploidies, which corresponds to the results of the above-mentioned study, bearing in mind that all our cases are of developmental age less than 12 weeks.

Due to the unreliable histological criteria for definitive diagnosis of partial molar pregnancy, additional molecular methods, such as molecular genotyping, can be of great help in doubtful cases. Conventional karyotyping or fluorescent *in situ* hybridisation (FISH) can be used to detect triploidy but cannot reveal the origin of the extra chromosomes. According to Kipp *et al.* (2010), the method of molecular genotyping has an advantage over other methods such as digital image analysis, flow cytometry, or fluorescence in situ hybridisation. Namely, this method may recognise paternal and maternal alleles and thus distinguish between androgenetic diploidy, androgenetic triploidy and biparental diploidy, which are actually characteristics of complete, partial mole and hydropic abortion [5]. In our study, molecular genotyping enabled distinction between androgenetic and gynogenetic triploidy in all 24 cases.

According to the published literature, diandric triploidies are probably more aggressive than digynic ones. For example, Seckl *et al.* (2000) studied 3000 partial hydatidiform moles and found three cases of choriocarcinoma, all of them after diandric triploid pregnancies [20]. However, there are insufficient data regarding the incidence of choriocarcinoma after digynic triploid pregnancy. Therefore it is unclear whether diandric and digynic triploidies have different malignant potential and whether their distinction should have a direct impact on the management of the patients [26].

Even though the risk of persistent trophoblastic disease after partial molar pregnancy is low, routine monitoring and follow-up of βHCG levels after evacuation is recommended [26, 27].

In conclusion, in routine practice the diagnosis of partial molar pregnancy cannot be based solely on histological evaluation of the products of conception. Additional molecular techniques should be used whenever possible in order to accurately determine the presence of molar pregnancy. This is important for the patients, not only because it gives them the comfort of knowing the reason for their spontaneous abortion, but more importantly because persistent trophoblastic disease, although rare, can still occur in cases of partial molar pregnancy.

The authors declare no conflict of interest.

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