New Insights in the Pathophysiology of Complete Hydatidiform Mole

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Abstract (250 words)

OBJECTIVE: The majority of complete hydatidiform moles (CHM) are detected on ultrasound examination by the end of the first trimester when they present as multiple sonolucent cysts. To better understand the pathophysiology of this unique placental pathology and improve its prenatal diagnosis and management we have reviewed the ultrasound features of CHM before the appearance of cystic changes.

STUDY DESIGN: We searched our database to identify all women diagnosed with a complete hydatidiform mole confirmed by histopathology who had an ultrasound examination before 9 weeks' gestation. We reviewed their ultrasound reports and all the corresponding images.

RESULTS: The study group included 39 women with a positive pregnancy test and vaginal bleeding, 36 of whom had at least two ultrasound examinations before 9 weeks' gestation. At the first scan (mean gestation age 7+1 weeks; SD 1.1), 29 out 39 (74.4%) of CHM presented as a heterogeneous hyperechogenic mass with or without gestational sac and the remaining ten (25.6%) cases as a regular 4-week gestational sac. Cystic molar changes became more obvious from the end of the second month of gestation.

CONCLUSION: The development of a CHM follows a well-defined pattern starting with a macroscopically normal gestation sac at 4 weeks, which transforms into a polypoid mass between 5 and 7 weeks of gestation. The hydropic changes of the villous tissue is progressive and rarely visible in utero on ultrasound before 9 weeks of gestation. These findings should allow an earlier diagnosis and assist in the management counselling of women with CHM.

Introduction

The first description of a molar pregnancy is attributed to Hippocrates around 400 BC [1,2] making this condition the oldest placental pathology known to medical science. Jan Baptist van Lamzweerde wrote the first monograph textbook on molar pregnancy entitled ‘Naturalis molarum uteri historia’ in 1687. William Smelie was the first to use the term “hydatidiform mole” in 1752 to describe “the bunch of grapes of different sizes” typical of this placental pathology. In 1827, Velpeau and Boivin first recognized hydatids as cystic dilations of chorionic villi but it was not until 1895 that Marchand described the proliferation of the trophoblast in tumours that develop after a hydatidiform mole [3]. Modern pathologists have characterized the classical or complete hydatidiform mole (CHM) as the generalized swelling of the villous tissue with diffuse trophoblastic hyperplasia but with no fetal tissue [4,5].

The prevalence of CHM is estimated at around 1 per 1000 deliveries in the UK and USA but varies around the world with a much higher prevalence in Asian populations [6]. The incidence of CHM is also 7-fold higher in adolescents and nearly twice as likely in women with advanced maternal age [7]. Women with CHM usually present with vaginal bleeding, uterine enlargement greater than expected for gestational age and abnormally high levels of serum human chorionic gonadotrophin (hCG), secondary to the trophoblastic hyperplasia [8,9]. With advancing gestation, several obstetric complications may develop including pre-eclampsia, hyperthyroidism, hyperemesis, anaemia and large ovarian theca-lutein cysts [8]. Thus it is not surprising that one of the first use of obstetric ultrasound

was for the prenatal diagnosis of CHM [10]. In the late 1960s, the first cohort studies demonstrated the high accuracy of ultrasound imaging in diagnosing CHM in the second trimester of pregnancy [11,12].

Like for its histopathologic features, the ultrasound features of CHM are now well established. They included a uterine cavity filled with multiple sonolucent cysts of varying size and shape bathed by maternal blood often described as "snow storm appearance" [13]. With the advent of high resolution imaging and the increased use of transvaginal ultrasound (TVS) in early pregnancy, the diagnosis of CHM has moved from second trimester to first trimester. From 9-10 weeks of gestation, the ultrasound diagnosis of CHM is accurate with up to 90% of the cases detected before the end of the first trimester [14-17]. However, there are no data on CHM before the appearance of cystic changes. The aim of the present study was to investigate the early-stages of development of CHM as evidenced by ultrasound imaging to better understand the pathophysiology of this unique placental condition.

Material and Methods

Our study group included women diagnosed with a CHM who had an ultrasound examination at $\leq$ 9 weeks of gestation at the Early Pregnancy Assessment Unit (EPAU) at University College London (North London) and King's College Hospital (South London) between 2005 and 2016. Women with multiple pregnancies and women with no stored ultrasound images for review were excluded from the study group. Demographic data were recorded, including maternal age and

ethnicity. The population studied included all cases of CHM who had an ultrasound examination in one the two EPAUs and had the diagnosis of hydatidiform mole confirmed by expert histopathologic examination. For the retrospective examination, all ultrasound images were anonymised. Local institutional review boards for each participating site approved the protocol and a waiver of consent.

All women presenting to the Early Pregnancy Assessment Unit (EPAU) with a positive pregnancy tests and symptoms of miscarriage are offered a detailed pelvic TVS as part of their medical assessment. All examinations are carried out by an experienced operator using a high-resolution transvaginal probe (Voluson 730 and E8 expert, GE, USA; Acuson XP/128, Siemens, Mountain View, CA, USA). All pregnancies are dated according to the last menstrual period (LMP) confirmed by gestational sac diameter and by fetal crown-rump length (CRL) in ongoing pregnancies.

Women presenting with ultrasound features indicating a missed or incomplete miscarriage are offered surgical evacuation of the uterus or expectant management with follow-up in line with our national EPAU guidelines. Women opting for conservative management are asked to attend one of the units after 14 days should their bleeding continue, and otherwise to check a urinary pregnancy test 2 to 3 weeks post-miscarriage. A subsequent ultrasound examination is performed if there is continuous vaginal bleeding or if the pregnancy test is still positive as previously described.[22] Women with early intrauterine pregnancies

(pregnancies of uncertain viability) are managed conservatively with a repeat ultrasound scan after 7-14 days.

Surgical management of miscarriage is recommended for all women with suspected molar pregnancy on TVS. All evacuated surgical tissue is sent for histological examination. All confirmed cases of molar pregnancy are registered with the regional gestational trophoblastic disease service at Charing Cross Hospital (London, UK) for follow-up. All cases of molar pregnancy diagnosed histologically are examined and cross-referenced with cases diagnosed on ultrasound and confirmed by a specialist pathologist from the regional referral centre.

Statistical analysis

The data were analyzed using StatGraphic-plus Version 3 statistical software package (Manugistics, Rockville, MD). Standard Kurtosis analysis indicated that all values were normally distributed and are therefore presented as mean and standard deviation (SD). A t-test was used to compare the means of gestational age at the time of the original diagnosis with that of the retrospective diagnosis. A P value of <0.05 was considered significant.

Results

During the time period of the study, 186 cases of molar pregnancies seen at one of the two EPAUs were confirmed by histopathology as molar pregnancies by the regional referral centre, including 105 CHM and 81 partial hydatidiform moles

(PHM). The study group included 39 women with confirmed CHM who presented for an ultrasound examination with vaginal bleeding ≤ 9 weeks of gestation. The maternal age ranged between 15 and 46 years with a mean 29 (SD 7.6) years. There were three (7.7%) women younger than 20 years old (adolescents) and four (10%) who were 40 years and older (advanced maternal age).

The mean gestational age at the first ultrasound examination was 7+1 weeks (SD 1.1; range 5.1-8.6). In two cases at 7+6 weeks and 8+3 weeks of gestation, respectively, a CHM was suspected at the first scan and the remainder were diagnosed with a missed, incomplete miscarriage or intra-uterine pregnancy of uncertain viability. In three of these cases, including the two suspected CHM cases, a surgical evacuation was performed immediately after the initial ultrasound examination. The remaining 36 women opted for conservative management and had a second ultrasound examination, 12-16 days after the first scan at a mean gestational age of 8+4 weeks (SD 1.4; range 7.1-11.2). In six (16.7%) of these cases, all ≥ 9 weeks of gestation, diffuse molar changes were documented on TVS and a surgical evacuation was performed. Twenty-five of the remaining women also opted for surgical evacuation immediately after the second scan and five women opted to continue with conservative management. These five women had a third ultrasound examination at 9+6 weeks (SD 1.6) which showed diffuse molar changes suggesting a CHM and had a surgical evacuation immediately after.

Table 1 summarises and compares the retrospective ultrasound data of the first and second scan. The most common (74.4%) ultrasound feature at the

first scan was the presence of a heterogeneous, mainly hyperechogenic (sonodense) mass, with or without an early gestational sac (Figure 1A and 1B). In the remaining ten cases, the diagnosis was an intra-uterine early gestational sac of less than 10 mm in mean diameter containing a collapsed secondary yolk sac in 4 cases (Figures 2A and 3A) suggesting an ongoing pregnancy of 4-5 weeks' gestation. At the second ultrasound examination, the distribution of the ultrasound findings had changed (Table 1) with the hyperechogenic structure, often presenting with a polypoid shape (Figures 2B and 3B) or containing focal molar changes (Figures 1C and 2C) and surrounded by sonolucent fluid spaces in 30 (83.33%) out of the 36 remaining cases. No gestational sac could be seen at that stage. In six of these cases, diffuse cystic changes were found on ultrasound indicating a CHM. In one of the five cases who had at third ultrasound examination and then presented with diffuse molar changes (Figures 3C and 4A), bilateral enlarged ovaries with theca-lutein cysts were noted. No other cases of multicystic ovaries were found in the entire cohort.

Overall, the preoperative original diagnosis after two or three ultrasound examinations identified a CHM in 20 (51.3%) out of the 39 cases whereas the retrospective review suggested a CHM in 29 (74.4%) cases at the first ultrasound examination. The mean gestational age at diagnosis of a molar pregnancy was significantly more advanced at the original ultrasound examinations compared to the retrospective review (9+3 weeks vs 8+3; $t= 4.27$; $P< 0.001$).

Discussion

The results of our study indicate that the development of a CHM in early pregnancy follows a well-defined pattern and that each stage is associated with specific ultrasound features. Access to at least 2 consecutive ultrasound examinations in over 90% of the cases, has allowed us to study the natural evolution of CHM and to identify the patterns of changes in the development of a CHM with advancing gestational age. In particular, this study shows for the first time, that a CHM starts at 4 weeks with what appears to be a morphologically normal gestational sac containing a chorionic cavity and sometime a secondary yolk sac.

The first structures visible on transvaginal ultrasound inside a normal gestational sac at the end of the 4^{th} week after the LMP are the chorionic cavity and the secondary yolk sac [18,19]. The fetal pole becomes visible on ultrasound at the end of the 5^{th} week of gestation and the fetal cardiac activity can be seen 34-35 days after the LMP, when the fetal pole is around 2-4mm. In the present study of CHM confirmed histologically, we did not observe the development of fetal structures and in particular no fetal heart activity was ever seen. Our previous finding of alpha-protein (AFP) inside the molar vesicles of CHM [20] and of yolk sac tissue in early CHM miscarriage [21], expressing AFP (unpublished observation) suggest that the echogenic structure found in the early gestational sac of CHM, in the present study, is most probably the secondary yolk sac. This suggest that in CHM, embryonic development stops soon after the formation of the germ disc i.e. just after the secondary yolk sac has started to form.

The formation of primitive placenta starts soon after implantation with the development of the primary villi [22]. These villi are made of projections of syncytiotrophoblast into the maternal decidua. Between days 13 and 15 post-conception, they are invaded first by cytotrophoblastic columns and extraembryonic mesenchyme to form secondary placental villi. Soon after mesodermal cells derived from the extraembryonic mesoderm invade the trabeculae, bringing with them the hemangioblasts from which fetal capillaries normally develop [19]. In CHM, we found that between 5 and 7 weeks of gestation, the villous tissue of the primitive placenta proliferates to form a heterogeneous mainly dense often polypoid mass. These ultrasound features correspond microscopically to dense mesenchymal tissue surrounded by hyperplastic trophoblast [21,23]. From the end of the second month of pregnancy, the progressive oedema of the villous mesenchyme gives the typical cystic molar changes found on ultrasound in all CHM from 9 weeks of gestation. These finding suggest that the syncytiotrophoblast in CMH allows for the normal transfer of water from the maternal blood intervillous. As the embryonic circulation never develops, this water accumulates progressively inside the villous mesenchyme creating the generalised hydropic macroscopic changes typical of CHM. A similar phenomenon has been observed in cases prolonged retention of placental tissue following embryonic demise where the trophoblast continues to perform its physiological biological functions for at least a week after the fetal heart has stopped [23].

During normal placentation, a subpopulation of trophoblast cells migrates from the deep surface of the cytotrophoblastic shell into the endometrium. These extravillous trophoblast (EVT) cells migrate simultaneously in a retrograde fashion down the lumens of the spiral arteries replacing the endothelium, and through the endometrial stroma [22,24]. In early pregnancy, the volume of the migrating endovascular EVT cells is sufficient to occlude, or plug, the terminal portions of the spiral arteries as they approach the basal plate [24,25]. It is the dissipation of these plugs towards the end of the first trimester that establishes the maternal circulation to the placenta [25]. We have previously observed that the EVT migration is almost completely absent in CHM and thus that the molar villous tissue is loosely attached to the uterine wall and the tips of the spiral arteries remains unplugged [21]. This can explain the presence of fluid spaces around the polypoid mass on ultrasound imaging at 5-7 weeks in the present study. The presence of moving fluid around the hydropic villi also suggest the precautious establishment of the intervillous circulation in CHM and can explain why women with CHM present with vaginal bleeding from very early in pregnancy.

The vast majority of CHM miscarry spontaneously during the first three months of pregnancy and it has been estimated that the incidence of hydatidiform moles is 1 per 41 early miscarriages [26]. With increasing access to EPAU with trained ultrasonographers, around 90% of CHM are now diagnosed and evacuated before the end of the first trimester [14-17]. From 9-10 weeks, ultrasound examination should correctly identify a uterine cavity filled with multiple

sonolucent cystic areas corresponding to hydropic molar villi in the vast majority of the cases. Before 9 weeks, the present data indicate that the majority of CHM are not hydropic and thus not as easily detectable on ultrasound. The main clinical implication of this finding is that if a CHM does not present with the typical molar changes at the initial ultrasound examination, ultrasonographers are likely to diagnose a missed-miscarriage or incomplete miscarriage and most women in our population then opt for conservative management.

Following uterine evacuation 15-20% of patients with a CHM develop persistent gestational trophoblastic neoplasia (GTN) requiring chemotherapy [8,9]. The risks of GTN development and need for second line chemotherapy is higher in Asian women [27] and lower in Hispanic women [28]. However, medical complications such as vaginal bleeding, anemia and clinical factors associated with post-molar GTN are more frequent among adolescents from south-America than from north-America [29]. In a series of 32 non-hydropic early histologically diagnosed CHM, eight (25%) women developed persistent GTN [30] suggesting all women with CHM, regardless of gestation at diagnosis, are at risk of subsequent GTN. These data highlight the importance of diagnosing molar pregnancy early and considering CHM in the differential diagnosis in women from higher risks groups.

Around two thirds of the women in our units diagnosed with a missed or incomplete-miscarriage opt for a conservative management and thus those who miscarry a CHM spontaneously may only be diagnosed with persistent GTN at a later stage. The gold standard for the diagnosis of a molar pregnancy is the

presence of trophoblastic hyperplasia on histological examination. Without histological confirmation, it is difficult to diagnose non-hydropic molar pregnancy based solely on ultrasound presentation. Nor can it be predicted from the clinical history. An epidemiologic study of 140 women presenting with GTD has identified a previous clinical miscarriage in 15% of the cases and no pregnancy in nearly 40% of the cases [32]. Only 3% of the women in this population had a previous CHM. This suggests that most pregnant women at subsequent risk of trophoblastic neoplasia are likely to be first seen with symptoms of miscarriage and be diagnosed with early pregnancy failure. Our data should enable the prenatal diagnosis of most cases of CHM before 9 weeks of gestation and assist in the management of this condition.

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Table 1: Retrospective ultrasound findings at the first (n= 39) and second (n= 36) scan in 39 cases of CHM.

Ultrasound features	N (%)
First ultrasound examination (mean GA 7+1 weeks)	
Heterogeneous hyperechogenic mass	29 (74.4)
Early gestational sac (<10 mm mean diameter)	10 (25.6)
Second ultrasound examination (mean GA 8+4 weeks)	
Heterogeneous hyperechogenic tissue with focal cystic changes	20 (55.6)
Heterogeneous hyperechogenic mass	10 (27.8)
Diffuse cystic changes	6 (16.7)

Figure legends

Fig.1: Transvaginal ultrasound views of the uterus in the same case: longitudinal (A) and transverse (B) views at 7+1 weeks showing a heterogeneous hyperechogenic mass (star); longitudinal at 9.1 weeks (C) showing focal cystic changes of approximately half of the mass (star).

Fig.2: Transvaginal ultrasound views of the uterus in the same case: at 4+5 weeks (A) showing a 5x3x7 mm gestational sac (GS) containing embryonic tissue and surrounded by normal decidual tissue; at 7+4 weeks (B) showing the hyperechogenic mass with a polypoid shape (stars) and peripheral sonolucent fluid spaces; at 8+1 weeks (C) showing focal cystic changes of approximately half of the mass (star).

Fig.3: Transvaginal ultrasound views of the uterus in the same case: at 6+2 weeks (A) showing a 3x4x3 mm collapsed gestational sac; at 8+2 weeks (B) showing a hyperechogenic mass of polypoid shape (stars) and peripheral sonolucent fluid spaces; at 10+1 weeks (C) showing diffuse molar changes.