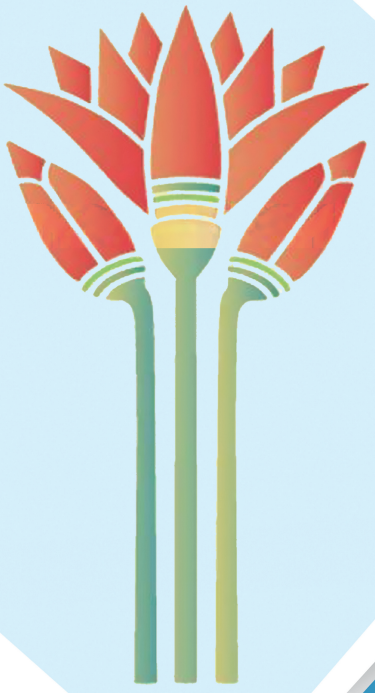
Studies on Gestational Trophoblastic Disease, with Emphasis on Improving Care in Egypt and Second Curettage in Low Risk Gestational Trophoblastic Neoplasia



Reda Hemida

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Studies on gestational trophoblastic disease
PhD Thesis
Reda Hemida

Studies on Gestational Trophoblastic Disease, with Emphasis on Improving Care in Egypt, and Second Curettage in Low Risk Gestational Trophoblastic Neoplasia.

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Studies on Gestational Trophoblastic Disease, with Emphasis on Improving Care in Egypt, and Second Curettage in Low Risk Gestational Trophoblastic Neoplasia.

Studies naar trofoblastziekten, met nadruk op verbeteren van de zorg in Egypte en op herhaald curetteren in laag risico persisterende trofoblast ziekte.

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List of abbreviations.

CM	**Complete mole**
PM	**Partial mole**
B-hCG	**B subunit of hCG**
GTD	**Gestational trophoblastic disease**
GTN	**Gestational trophoblastic neoplasia**
MTX/FA	**Methotrexate/folinic acid**
EMA/CO	**Etoposide, methotrexate, actinomycin-D, cyclophosphamide, vincristine.**
FIGO	**International Federation of Obstetrics and Gynecology**
WHO	**World Health Organization.**
LR	**Low-risk**
HR	**High-risk**
IHC	**Immunohistochemistry**
US	**Ultrasound**
CT	**Computed Tomography**
MRI	**Magnetic Resonance Imaging**
D&C	**Dilatation and curettage**
MVA	**Manual vacuum aspiration**

Chapter 1

General Introduction and Overview: Objectives and Outline of the Thesis

Introduction

1. Definition and prevalence of GTD

Gestational trophoblastic disease (GTD) consists of a spectrum of interrelated conditions arising from the placenta [1].The malignant form is known as gestational trophoblastic neoplasia (GTN) and trophoblastic tumor. Histologically, GTD is classified into benign forms of complete and partial hydatidiform moles and malignant forms of invasive moles, gestational choriocarcinoma, rare placental site trophoblastic tumors (PSTT), and epithelioid trophoblastic tumor (ETT) [2]. The incidence of GTD differs according to the geographic location, which has been attributed, at least in part, to racial or ethnic differences [3]. The reported incidences are 1/125 live-births in Taiwan, 2/1000 pregnancies in Japan and South East Asia, 1/1500 in United States, and 1/1000 in Europe [4].

2. Hydatidiform mole

2.1. Pathogenesis of molar pregnancy

Molar pregnancies are subdivided histopathologically and genetically into complete mole (CM) and partial mole (PM). CMs are without evidence of fetal tissue—diploid and from androgenic origin. Up to 80% of CMs arise from the fertilization of an ovum by a single sperm where the sperm duplicates after fertilization, whereas 20%–25% of CMs arise from fertilization of an ovum by two different sperms [1]. Of PMs, 90% are triploid in origin with one set of maternal haploid genes and two sets of paternal haploid genes. In most cases, PMs occur succeeding dispermic fertilization of an ovum; 10% PMs are tetraploid or mosaic in origin. In case of PMs, there is evidence of fetal tissue or fetal red blood cells [5]. Theca Lutein cysts may be present in 20%–40% of patients with CMs [6]. Malignant sequel occurs in 20% and <5% of patients with CMs and PMs, respectively [7]. Immunostaining with P57 is an established method to distinguish between CMs and PMs and non-molar pregnancies [8]. P57 is expressed exclusively by maternal chromosomes so that immunostaining is negative in CMs and positive in PMs and non-molar pregnancies [9].

2.2. Human chorionic gonadotropin

GTD produces human chorionic gonadotropin (hCG) is a disease-specific tumor marker. It is easily measured quantitatively in urine and blood, and its level corresponds with the disease severity. hCG

is a placental glycoprotein comprising two dissimilar subunits: α-subunit resembling that of the pituitary glycoprotein hormones and β-subunit that is unique to placental production. hCG exists in many forms, including at least six crucial variants, which are detected in serum, as follows: hyperglycosylated, nicked, absent C-terminal of β subunit, free β subunit, nicked free β subunit, and free α subunit. In GTD, hCG molecules differ from those in normal pregnancy; they are more heterogeneous and degraded in GTD. Considering this, an assay that can recognize all main forms of hCG and its multiple fragments should be used for following up on patients with GTD [10]. Nowadays, majority of institutions perform rapid automated radiolabeled monoclonal antibody sandwich assays measuring distinct mixtures of hCG-related molecules [11]. GTD is usually accompanied with markedly elevated hCG values higher than those during normal pregnancy. Nearly 50% patients with CMs have pre-evacuation hCG levels >100,000 mIU/mL [12].

2.3. Diagnosis of molar pregnancy

Typically, GTD is diagnosed during first trimester. The most common symptom of complete hydatidiform mole (CHM) is vaginal bleeding, whereas other symptoms include hyperemesis gravidarum, uterine enlargement more than the expected gestational age, absence of fetal heart tones, high values of hCG compared with gestational age, and pregnancy-induced hypertension [13].

Nearly 40%–60% of complete and partial molar pregnancies are detected by transvaginal ultrasound. However, histologically, 10% of suspected molar pregnancies based on ultrasonography appear to be non-molar hydropic abortions [14]. Therefore, histological examination of any material related to non-viable pregnancy should be performed [15]. Recently, a CM has been progressively diagnosed; however, to our knowledge, CM has not been reported to be associated with change in the development of GTN [16].

2.4. Treatment of molar pregnancy

Molar pregnancy is treated by suction evacuation under general anesthesia, but local or regional anesthesia can also be used. Suction evacuation is performed after serial Hegar dilatation of the uterine cervix [17]. Ultrasound guidance facilitates complete evacuation of the uterus. Intravenous oxytocin is used after the dilatation of the cervix and should be continued for several hours postoperatively to reduce uterine bleeding. Anti-D immunoglobulin should be administrated for Rh-negative patients after evacuation, although fetal red blood cells should not be present in a CM due to the expression of Rhesus D factor on trophoblast [18]. Trophoblastic embolization may occur

after molar evacuation, which is the main cause of respiratory distress. There are also other different causes, such as anemia, hyperthyroidism, and iatrogenic fluid overload [19].

2.5. Monitoring and contraception

Following evacuation, it is mandatory to monitor all patients to diagnose and treat malignant sequel. Serial consecutive quantitative serum hCG levels should be performed. It is preferred to do so in a single laboratory using the same measurement kit. Ideally, serum hCG levels should be evaluated after 48 hours of evacuation, every 1–2 weeks while elevated, and at monthly intervals for subsequent 6 months. Patients who require over 56 days to reach normal hCG value have ten-fold increased risk of developing GTN after hCG normalization [20]. Regression curves have been designed to determine the pattern of hCG during follow-up [21].

The Dutch guidelines recommend no further follow up after first establishing a normal value of hCG [22]. Moreover, the data of a recent meta-analysis supports decreasing hCG monitoring from six to three months postnormalization in CM and performing only one confirmatory check after a month in PM [23].

Hormonal contraception is recommended while monitoring hCG values. Reported studies support using hormonal contraception as it does not intensify risk of post-molar GTN or delayed hCG normalization [24]. Contraception is allowed in Islamic laws for the sake of mother's health and welfare.

Pregnancies following molar evacuation (after 6 months of contraception) are usually normal, although pregnancies conceal the value of monitoring hCG levels, which may result in a delayed diagnosis of post-molar malignancy [25].

2.6. Prophylactic chemotherapy

Prophylactic chemotherapy decreases the risk of progression to GTN in women with CMs who are at a high risk of malignant transformation; however, currently, there is limited evidence supporting prophylactic chemotherapy due to poor methodological quality and small size of included studies. As prophylactic chemotherapy may increase drug resistance, delay treatment of GTN, and expose women to toxic side effects, this practice should not to be recommended unless in a prospective study [26]. Additionally, the chemotherapy does not eradicate the need for post-evacuation follow-up.

2.7. Diagnosis of post-molar GTN

Diagnosis of GTN includes an increase in hCG levels after evacuation of HMs and/or histologically diagnosed as gestational choriocarcinoma or invasive mole, ETT, or PSTT; it resents clinical or radiological evidence of metastasis. To diagnose post-molar GTD, different modalities of hCG-interpreting criteria have been conducted. The international federation of gynecologist and obstetricians (FIGO) standardized hCG criteria for this purpose [27]. The following criteria were submitted by FIGO:

1- An hCG level plateau of 3 values plus or minus 10% recorded over 3-week duration (days 1, 7, and 21).

2- An hCG level rise >10% of 3 values recorded over 2-week duration (days 1, 7, and 14).

3- Persistence of detectable hCG for >6 months after molar evacuation.

The Charing Cross Trophoblastic Disease Center (London, United Kingdom) recommends immediate start of chemotherapy in case serum hCG concentration is ≥20000 mIU/Ml after four or more weeks of uterine evacuation, considering the increased chance of developing GTN in such patients. This recommendation was adopted by the European Organisation for the Treatment of Trophoblastic Disease (EOTTD) and many countries worldwide. However, this recommendation has not been adopted by FIGO [20].

2.8. Recurrent HM

Recurrent HM (RHM) is described as the occurrence of at least two HMs in the same patient. Fifty to eighty percent of patients with RHMs have bi-allelic pathogenic variants in NLRP7 or KHDC3L. However, not all genotypic types of the moles are yet identified [28].

3. Gestational trophoblastic neoplasia (GTN)

3.1. Classification

Table 1 represents a revised FIGO scoring system including various patient characteristics that affect the response to chemotherapy. The following factors contribute to determining FIGO prognostic score: patient's age, previous pregnancy, duration of disease, pretreatment hCG level,

site and number of metastases, size of the largest tumor, and exposure to prior chemotherapy [2]. However, histopathologic diagnosis is not included as a factor.

Each item is scored 0–4; after sum of these risk scores; ≤6 is defined as low-risk factor, whereas ≥7 is high-risk [27]. Moreover, corresponding to a combined anatomic staging and scoring system, FIGO defines low-risk GTN as non-metastatic (stage I) and metastatic (stages II and III) disease with a prognostic score of <7. Nevertheless, FIGO stage IV—or any stage with a World Health Organization (WHO) score of >7—indicates high risk of resistance to single-agent chemotherapy, elevated risk of recurrence, and the necessity for combination chemotherapy to get the best outcome [29].

Table 1. Revised FIGO Scoring System [27]

FIGO Score	**0**	**1**	**2**	**4**
Age	≤39	>39	—	—
Antecedent Pregnancy	Hydatidiform mole	Abortion	Term pregnancy	—
Interval from index pregnancy (months)	<4	4—6	7—12	>12
Pretreatment hCG level (mIU/mL)	<1,000	1,000—10,000	>10,000—100,000	>100,000
Largest tumor size including uterus (cm)	3—4	5	—	—
Site of metastasis	Lung, vagina	Spleen, kidney	Gastrointestinal tract	Brain, liver
Number of metastasis identified	0	1—4	4—8	>8
Previous failed chemotherapy	—	—	Single drug	2 or more drugs

GTN is highly responsive to chemotherapy. Low-risk GTN can be cured with single-agent chemotherapy with either methotrexate (MTX) or actinomycin-D (Act-D) in 90% of cases; multi-agent chemotherapy is required in 10% [30]. For patients with high-risk GTN, multi-agent chemotherapy is the primary treatment of choice [31]. Worldwide, the most commonly used regimen for such patients comprise etoposide, MTX, Act-D, cyclophosphamide, and vincristine (EMA/CO combination) [32].

It has been reported that high hCG levels [33], presence of metastatic disease [34], CM [30], high-risk FIGO score [35], increasing neo-angiogenesis [36], and patients aged above 40 years [37] are associated with increased risk of initial chemotherapy resistance and long time to achieving remission in low-risk GTN patients.

In treating PSTT and ETT, the FIGO score has no rule because single-agent chemotherapy should not to be the first therapeutic choice for these rare tumors [38].

Adjuvant surgical treatment of GTN can be implemented to decrease tumor load in the uterus at the start of treatment; this leads to reduced need for chemotherapy when future pregnancy is no longer desired, control tumor hemorrhage, and/or remove resistant/persistent disease in the uterus or at metastatic sites [39]. While treating ETT and PSTT, surgical intervention is mainstay as these tumors are often limited to the uterus and less sensitive to chemotherapy [40].

3.2 Clinical and pathologic considerations

The clinical presentation of post-molar GTN is far more essential than the histologic or radiological evidence in determining its management and prognosis [41]. The term invasive mole describes the disease confined to the uterus and is characterized by the presence of edematous chorionic villi with trophoblastic proliferation that invades the myometrium. Majority of patients are clinically diagnosed and not confirmed histologically. Dilatation and curettage should be avoided to prevent morbidity or mortality caused by uterine perforation of the soft uterus during the procedure [42]. Gestational choriocarcinoma develops early distant metastasis in the vagina, lung, liver, and brain; thus, chemotherapy should be started in a well-timed manner to avoid bleeding complications at metastatic sites [43]. PSTT is characterized by the absence of villi and presence of intermediate trophoblastic cells [40]. The number of syncytiotrophoblastic cells is less in PSTTs, with subsequently decreased levels of secreted hCG. Additionally, PSTTs are lesser sensitive to chemotherapy than other types of malignant GT cells [44].

Most PSTTs follow non-molar gestations. PSTTs often exhibit diffused immunostaining with intermediate trophoblastic markers, such as human placental lactogen and Ki67 [45]. ETT is a rare variant of PSTT that simulates carcinoma. Depending on morphologic and histochemical aspects, ETT develops from the neoplastic transformation of chorionic-type intermediate trophoblasts. Majority of the cases occur many years after a full-term delivery [46]. Both PSTT and ETT produce low levels of hCG.

3.3 Management of GTN

As soon as the diagnosis of malignant GTD is suspected, metastasis should be evaluated. Besides history and physical examinations, the following laboratory studies should be performed: blood type and antibody screen, complete blood count, clotting studies, liver and renal studies, and the determination of pre-therapy hCG level. The following radiographic studies are recommended: chest X-ray or computed tomography (CT) scan of the chest and pelvic ultrasonography. Rarely, MRI scan or abdominopelvic CT with contrast is recommended. CT scan of the brain is needed in symptomatic patients or in cases of pulmonary metastasis [27,47]. The role of positron emission tomography (PET) in evaluating metastatic GTN has not yet been well established. The available information suggests that PET does not add value to GTN staging, whereas conventional imaging work-up is lesser expensive and more widely available than PET [6].

Management of low-risk non-metastatic GTN

Generally, low-risk (LR) non-metastatic GTN is treated with single-agent chemotherapy using either MTX or Act-D. There are different MTX regimens used as initial treatment for patients with LR (50 mg fixed dose, 50 mg/m2 of body surface area, or 1 mg/kg of body weight on days 1, 3, 5, and 7, with or without folinic acid rescue, 0.4 mg/kg on days 1–5, and 30–50 mg/m2 once weekly). There are also different Act-D regimens used, including 10–13 mcg/kg on days 1–5 and 1.25 mg/m2 biweekly. This makes it difficult, with the data available, to actually evaluate the best initial treatment for low-risk GTN [48]. MTX is excreted entirely by the kidneys and can result in hepatic toxicity; thus, patients should test their normal renal and liver functions before each treatment. Hematologic indices should be carefully monitored during chemotherapy.

According to the updated Cochrane systematic review [48], Act-D is more likely to achieve primary cure in women with low-risk GTN and less likely to result in treatment failure than MTX regimen. However, Act-D is associated with greater risk of severe adverse events and costlier than MTX regimen.

Chemotherapy should be continued until normal hCG levels are achieved, consequently additional two courses should be administrated after the first normal hCG value record. However, after a retrospective analysis of LR patients treated in the Netherlands and UK, Lybol et al. [49] concluded that three courses of consolidation chemotherapy are better than two in treating low-risk GTN to decrease the risk of disease relapse.

In selected cases, a hysterectomy is an effective way to decrease or eliminate tumor bulk. As a first line of management, hysterectomy should be considered in older patients with localized disease and no desire to preserve fertility and those presenting with chemotherapy resistance. For patients with widespread distant metastasis, the value of hysterectomy exists in removal of chemotherapy-resistant tumor bulk with favorable effect on survival [50]. After hysterectomy, chemotherapy is mandatory in women with metastasis until normal hCG levels are achieved.

Second uterine curettage

The role of second uterine curettage as a single or additional treatment in the management of postmolar GTN is unclear. Previous retrospective studies have found widely differing cure rates varying 9%–80% [51,52]. A debulking effect of second uterine curettage has been reported by two retrospective analyses that reported that few chemotherapy courses were required to reach undetectable serum hCG levels after second curettage [51,53]. This reduction is related to serum hCG level and the presence or absence of myometrial invasion and distant metastases.

Recently, two prospective observational studies were published. First, a small prospective pilot study reported cure in 10 of 12 patients after second curettage in post-molar GTN [54]. The second study, performed by the Gynecologic Oncology Group, reported cure rates of 40% for low-risk non-metastatic GTN using second uterine curettage as a single treatment [55]. The disadvantages of second uterine curettage include complications such as uterine perforation, infection and bleeding, and delay in starting chemotherapy when post-curettage hCG levels fail to normalize [51,55].

Second curettage is not recommended by The American College of Obstetricians and Gynecologists because it does not often result in the remission of hCG levels or offer help in the management of GTN; however, it can result in uterine perforation and hemorrhage [42].

Management of low-risk metastatic GTN

Women with FIGO risk score <7, metastatic disease, and any high-risk clinical factors are still considered to have a low-risk disease.

Treatment can be successfully achieved with initial single-agent regimens. This therapy is often continued for 5 days of MTX treatment or intravenous actinomycin-D recycled at 14-day interval. Most patients with low-risk metastatic GTD can be cured using conventional single-agent chemotherapy [56]. Hysterectomy, in additional to chemotherapy, decreases the amount of

chemotherapy needed to achieve remission in these patients [31]. Similar to the treatment of non-metastatic GTD, two cycles of chemotherapy should be given after the first normal hCG value. The overall complete remission rate is up to 100% [56].

Management of high risk metastatic GTN

Patients with FIGO risk score of 7 or more are considered to have a high-risk disease. They require multi-agent chemotherapy. Surgery and/or radiation are often incorporated in the treatment [57].

Aggressive treatment by multi-agent chemotherapy for these women is an integral part of the management. Nearly 25% patients with high-risk metastatic GTN have refractory disease, relapse, or extensive metastatic disease (FIGO stage IV, score > 12) and require recognition of chemotherapy-resistant sites for surgical resection, central nervous system irradiation, and/or alternate therapeutic regimens. The most commonly used chemotherapeutic regimens include EMA/CO, EMA-EP (etoposide, MTX, Act-D, etoposide, cisplatin), and TE/TP (paclitaxel, etoposide, paclitaxel, cisplatin), which results in 75%–80% response rate [58].

Regarding ultra-high risk group defined as FIGO score >12; patients should immediately be referred to a GTD Centre. Imaging should be performed if not recently done (contrast CT-chest/abdomen, MRI brain, MRI pelvis). Low-dose induction EP for 1-3 cycles should be considered depending on the clinical condition followed by EP/ EMA or EMA/CO. After normalization, consolidation courses should be given for 8 weeks according to local GTD Centre advice [59].

The management of brain metastasis is controversial. Radiation therapy is concurrently used with chemotherapy to limit acute hemorrhagic complications occurring from these metastases. Systemic chemotherapy combined with brain irradiation is successful in controlling brain metastasis, with cure rates up to 75% [60]; intrathecal MTX infusion without brain irradiation has similar remission rates [61].

In the hope of eradication of all viable tumors, chemotherapy should be continued till hCG values normalize, followed by at least 2–3 courses of maintenance chemotherapy.

3.4 Follow-up

Following the remission of hCG values, patients with malignant GTD should undergo serial testing of hCG levels at 2-week intervals for the first 3 months of remission and after that at 1-month intervals for 1 year.

In the follow-up of GTN, most relapses are noted to occur within the first 12 months after completion of chemotherapy; biannual measurement of β-hCG for 5 years is usually sufficient [20].

3.5 Fertility after treatment of GTN

Most patients with GTN are in reproductive age group, and preserving fertility is an important issue to them. Chemotherapy can affect ovarian function, and the extent of gonadotoxic effect depends on the type of chemotherapy, dose and schedule of treatment, and patient's age [62]. Major concerns related to chemotherapy are possible infertility, risk of premature ovarian failure, and the mutagenic and teratogenic effects of chemotherapy that can affect subsequent pregnancy outcomes.

The psychological effects of GTN should be considered, especially in younger patients; questions about infertility and premature menopause increase distress and decrease patient compliance in follow-up [63]. Nevertheless, the obstetric outcomes of those who conceive after chemotherapy are similar to those of the general population, but patients should be advised not to get pregnant for at least the next 1 year to avoid any misinterpretation of hCG results and possible harmful effects of chemotherapy on the ovaries and fetal outcome. Nonetheless, if patients conceive within that 1 year, they can be reassured that overall outcome is favorable and there is no need to terminate pregnancy [64].

4. Objectives and outline of the thesis

This thesis aims to investigate several aspects of the diagnosis and treatment of GTN with special focus on the effect of second uterine curettage on the number of chemotherapy courses required to achieve hCG normalization.

Chapter 1 presents a general introduction that aims to provide literature review regarding classification, diagnosis, and modalities of the treatment of GTD. The chapter ends with objectives of the thesis. In *chapter 2*, we investigated incidence, prognosis, and outcome of GTD in Lower Egypt. Until now, the data of incidence and outcome of GTD in Egypt were scarce, which may be due to poor registration of cases. We conducted a prospective 1-year study aimed to address the

incidence and outcome of GTD at Mansoura University Hospital, which serves most of patients from Lower Egypt after the development of specialized GTD clinic with a strict registration system.

So far, globally, no consensus guidelines are available for treating GTN in patients who are ≥ 40 years old. Treatment of such cases depends mainly on expert opinions. In chapter 3, we performed a multi-center retrospective analysis on the clinical outcomes of different treatment strategies in patients aged 40 years or above. For this, we analyzed the data retrieved from five centers in five countries.

Subsequently, we aimed to investigate the effect of second uterine curettage on the number of chemotherapy courses needed to achieve hCG normalization in low-risk GTN patients. We performed a single-center randomized phase II trial in patients with low-risk post-molar GTN to evaluate the impact of second curettage on the number of chemotherapy courses, need of second-line chemotherapy, relapse rate, and complications, results of which are presented in chapter 4.

It is crucial for us to share our experience of performing this randomized study. In chapter 5, our considerations have been mentioned. We address benefits and obstacles of collaboration between Western and non-Western countries in conducting high-quality scientific researches.

We explored the reproductive outcomes achieved after fertility-preserving treatment of premalignant and malignant gynecologic tumors, including GTN. GTN usually occurs in reproductive age where the fertility of patient is keened by family and physician. As described in chapter 6, a retrospective study was conducted to find the reproductive outcomes after treatment of premalignant and early gynecologic malignancies, including 34 cases of GTN.

We investigated the prevalence of GTD after histopathologic examination of specimens of pregnancy termination and post-abortive bleeding. We hypothesized that some cases of GTD were misdiagnosed as miscarriage on clinical basis, and the diagnosis of GTD has been settled only after histopathologic examination. In chapter 7, we conducted histopathological review of 640 specimens of contents of uterine evacuation after pregnancy termination and post-abortive bleeding.

We planned to determine the most specific histopathological and immunohistochemical features required for accurate diagnosis that can reliably predict the clinical behavior of molar pregnancy as presented in chapter 8. Although the morphological characteristics of products of conception specimens, including molar pregnancies, are well described, substantial histopathological similarities are observed between different entities, especially in cases of early pregnancies. Furthermore, to our knowledge, there are no criteria for predicting cases with progression to persistent GTD.

We investigated the mutational status in a patient with six recurrent molar pregnancies who was managed in the GTD clinic of Mansoura University. *NLRP7* sequencing was performed, and mutation analysis revealed a novel mutation in *NLRP7* as described in *chapter 9.*

Chapter 10 presents the general discussion of different studies presented in this thesis and their impact on the decision of the management of patients with low-risk GTN and future perspectives.

Chapter 11 presents the summary of the thesis in English, Dutch, and Arabic languages.

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Chapter 2

Incidence and Outcome of Gestational Trophoblastic Disease in Lower Egypt

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Abstract

Background. Gestational trophoblastic disease (GTD) defines a spectrum of proliferative disorders of trophoblastic epithelium of the placenta. Incidence, risk factors, and outcome may differ from a country to another.

Objective. To describe incidence, patients' characteristics, treatment modalities, and outcome of GTD at Mansoura University which is a referral center of Lower Egypt.

Methods. An observational prospective study was conducted at the GTD Clinic of Mansoura University. The patients were recruited for 12 months from September 2015 to August 2016. The patients' characteristics, management, and outcome were reported.

Results. We reported 71 clinically diagnosed GTD cases, 62 of them were histologically confirmed, 58 molar (33 CM and 25 PM) in addition to 4 initially presented GTN cases. Mean age of the studied cases was 26.22 years ± 9.30SD. Mean pre-evacuation hCG was 136170 m.i.u/ml ±175880 SD. Most of the cases diagnosed accidentally after abnormal sonographic findings (53.2%). Rate of progression of CM and PM to GTN was 24.2% and 8%, respectively.

Conclusion. The incidence of molar pregnancy and GTN in our locality was estimated to be 13.1 and 3.2 per 1000 live births respectively. We found no significance between CM and PM regarding hCG level, time to hCG normalization, and progression rate to GTN.

Key words. Molar pregnancy; incidence; outcome.

Introduction

Gestational trophoblastic disease (GTD) defines a spectrum of proliferative disorders of trophoblastic epithelium of the placenta [1]. GTD was classified histologically into benign forms of complete and partial hydatidiform moles and malignant forms of invasive moles, gestational choriocarcinoma, placental site trophpoblastic tumors (PSTT), and epithelioid trophoblastic tumors [2]. The incidence of gestational trophoblastic disease differs according to geographic distribution. The highest reported incidence was 1/125 live births in Taiwan, while 2/1000 pregnancies in Japan and South East Asia, 1/1500 in United States and 1/1000 in Europe [3]. However, underestimation of the molar pregnancy incidence may occur if the products of conception are not routinely subjected to histological examination and if the registry system is not developed [4]. Risk factors of molar pregnancy include genetic, racial [5], extremes of maternal age [6,7], dietary and nutritional factors [8].

Hydatidiform moles typically are diagnosed during the first trimester [9]. Abnormal vaginal bleeding is the commonest symptom. Other signs and symptoms include hyperemesis gravidarum, oversized uterus, absent fetal heart pulsations, pregnancy induced hypertension and abnormally high levels of hCG [10]. By ultrasound, molar tissue is usually identified as a diffuse mixed echogenic pattern replacing the placenta (snowstorm), produced by villi with intervening intrauterine blood clots [11]. Treatment of molar pregnancy is by suction evacuation with a soft plastic cannula [12] with ultrasound control. Following evacuation, it is mandatory to monitor all patients to diagnose and treat malignant progression.

Post-molar GTN is typically diagnosed in patients with serum B-hCG raised, plateau, or persistent beyond 6 months of molar evacuation [13]. GTN are categorized into low or high risk according to the International Federation of Gynaecology and Obstetrics (FIGO) staging and modified World Health Organization (WHO) risk-factor scoring system [14]. Patients with FIGO stages I–III with a score of 0–6 are categorized as low-risk GTN while either FIGO stage IV or any stage with WHO score $\geq$7 are classified as high risk [15]. GTN is well known to be highly responsive to chemotherapy. Low-risk GTN is cured with single-agent chemotherapy with either methotrexate or actinomycin-D in 90% of the cases [16]. High-risk GTN is treated with combination chemotherapy to optimize outcome [17,18].

Since the incidence, patients' characteristics, treatment modalities, and outcome of gestational trophoblastic disease may differ from country to another; we conducted this prospective study to describe our early experience in Gestational Trophoblastic Clinic, Mansoura University, Egypt.

Methods

An observational prospective study was conducted at the GTD Clinic of Mansoura University Hospitals, Mansoura, Egypt. Mansoura University Hospital provides tertiary healthcare for most of the Delta region of Egypt, with a population of about 12 millions. The patients were recruited for 12 months (from September 2015 to August 2016), followed by 6 months so that the follow up was at least 6 months for all patients.

Participants

Inclusion criteria:

The current study included both molar pregnancies and GTN. Molar pregnancies were diagnosed clinically and based on ultrasound criteria with an abnormally high hCG levels. Patients presented by gestational trophoblastic neoplasia included postmolar GTN (with serum β-hCG raised, plateau, or persistent beyond 6 months of molar evacuation) or cases with histological evidence of choriocarcinoma, invasive mole, PSTT, and epithelioid trophoblastic tumors.

Exclusion criteria:

Histologic confirmation of "products of conception" after suction evacuation and patients who refused to participate in the study.

Collected patient variables included the age, the body mass index, parity, gestational age, uterine size in weeks, sonographic findings, serum β-hCG, lung metastasis, and medical diseases were recorded. To get a rough estimate of prevalence of GTD in our Hospital; the included cases were compared to the whole number of live births in Mansoura University Hospitals.

Treatment of molar pregnancy:

1. Pre-operative preparation: routine laboratory tests, β-hCG, chest X-ray and anaesthetic consultation were performed.

2. The patients were treated by suction evacuation using a soft plastic cannula, guided by ultrasonography under short acting general anaesthesia. After dilation of the cervix; Oxytocin 5 IU ampoule (Syntocinon, Novartis, Egypt) was given in 500 ml saline infusion in case of severe uterine bleeding during suction evacuation. After the procedure was completed; Ergometrine 0.2 mg ampoule (Methergine, Novartis, Egypt) was given intramuscular to reduce uterine bleeding. Prophylactic broad spectrum antibiotic was given. The patient was discharged 48 hours after evacuation.

3. Follow up. The patients were followed by serum β-hCG weakly till 3 negative results (below the reference range of 5 mIU/mL). Subsequently, hCG was checked monthly for 6 months to insure that the hCG levels remained undetectable. During the post evacuation management, the patients were under the umbrella of contraception; preferably combined oral contraception pills.

Diagnosis, staging, and risk factors for gestational trophoblastic neoplasia:

Progression to GTN was diagnosed using the FIGO, 2002 criterion [15]: hCG levels rising (more than 10%) for three consecutive weeks, plateaued for four weeks or persistent beyond 6 months. Patients with a histological diagnosis of any of the malignant forms or metastases detected during post-molar follow-up were also classified as GTN cases. GTN was staged according to the FIGO, 2002 criteria and classified into low or high risk according to modified WHO scoring system. The diagnosed cases as GTN were discussed in the tumor board meetings to receive chemotherapy and possibility of surgical interference.

Ethical considerations.

Oral and written consent was taken from the patients. The study was approved by the institutional review board (IRB) of Faculty of Medicine, Mansoura University (number: MS/16.01.01).

Statistical analysis

Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square and Fischer exact tests. Continuous variables were presented as mean ± SD (standard deviation) for parametric data and median (Min-Max) for non-parametric data. The two groups were compared with Student t test for parametric data and Mann Whitney test for non-parametric data. *P* values were considered statistically significant when $p < 0.05$.

Results

Between 1st of September 2015 till the 31st of August 2016, 71 clinically diagnosed patients as GTD were included in the study. Sixty-two were confirmed histologically to have GTD (58 cases of molar pregnancy and 4 cases of GTN). The total number of life births in MUH during the same period was 4398 thus the incidence of molar pregnancy and GTN in MUH is estimated to be 13.1 and 3.2 per 1000 live births respectively. In the same period of time, the number of live births in Dakahlia governorate was 155,962 representing a population-based incidence of molar pregnancy

and GTN 0.37 and 0.09 per 1000 live birth respectively. The mean age of the cases with molar pregnancy was 26.22 ± 9.30 years with 16.1% of the cases are less than 18 years old and 12.9% are 40 years old or more. We reported one case with familial recurrent hydatidiform moles where CM was diagnosed in her 2 sisters. Genetic study was done for the sisters and revealed a mutation of NLRP7.Other sociodemographic criteria were shown in table (1). The clinical presentation of the studied cases is demonstrated in table (2), as can be noticed; thirty-three cases (53.2%) were accidentally diagnosed by ultrasound during routine antenatal care visits. Chest X-ray was free for all diagnosed cases. The sensitivity of ultrasound in diagnosis of molar pregnancy was calculated to be 87% while the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of ultrasound in differentiating complete and partial moles is presented in table 3 which were found to be 96.7%, 73.6%, 85.2%, 93.3%, and 87.7% respectively.

Table (1): Demographic data of newly diagnosed cases of GTD in one year.

Variables	**The study group (n=62)**	
	No	%
Age (years)		
<18	10	16.1%
18 to <40	44	71.0%
≥40	8	12.9%
Mean ± SD	26.22±9.30	
(Min.-Max.)	16-52	
BMI*		
Mean ± SD	24.55±4.01	
(Min.-Max.)	19-35	
Parity		
Nullipara & Primi para	33	53.2%
Multipara	29	46.8%
Mode of delivery (n=42)		
Cesarean section	24	57.1%
Vaginal delivery	18	42.9%
History of miscarriage	22	35.5%
Medical diseases	**n=23**	**37.1%**
Anemia	15	65.2
Asthma	2	8.7
Diabetes	2	8.7
Hypertension	1	4.3
Hyperthyrodism	1	4.3
Hypothyrodism	1	4.3
Deep venous thrombosis	1	4.3
Positive family history	1	1.6%

*****BMI: Body Mass In

Positive family history**: Recurrent CHM was diagnosed in her 2 sisters.

Table (2): Clinical presentation of the studied cases:

Clinical presentation (n=62)	**No**	**%**
Diagnosed by ultrasound	33	53.2
Vaginal bleeding	21	33.9
Hyperemesis gravidarum	4	6.5
Lower abdominal pain with pregnancy	2	3.2
Stoppage of menstrual cycle above 50 years old	1	1.6
Early onset Preeclampsia*	1	1.6

(*) One patient was admitted in the Neurology department because of convulsive fits and was diagnosed to have early onset preeclampsia with molar pregnancy.

Table (3): Accuracy of ultrasound in differentiating complete and partial moles.

U/S Diagnosis		**Histopathology**		**Total**
		Complete mole	**Partial mole**	
	Complete mole	29	5	34
	Partial mole	1	14	15
	Total	30	19	49

Sensitivity: 96.7% **Specificity:** 73.6% **PPV:** 85.2%

NPV: 93.3% **Accuracy:** 87.7%

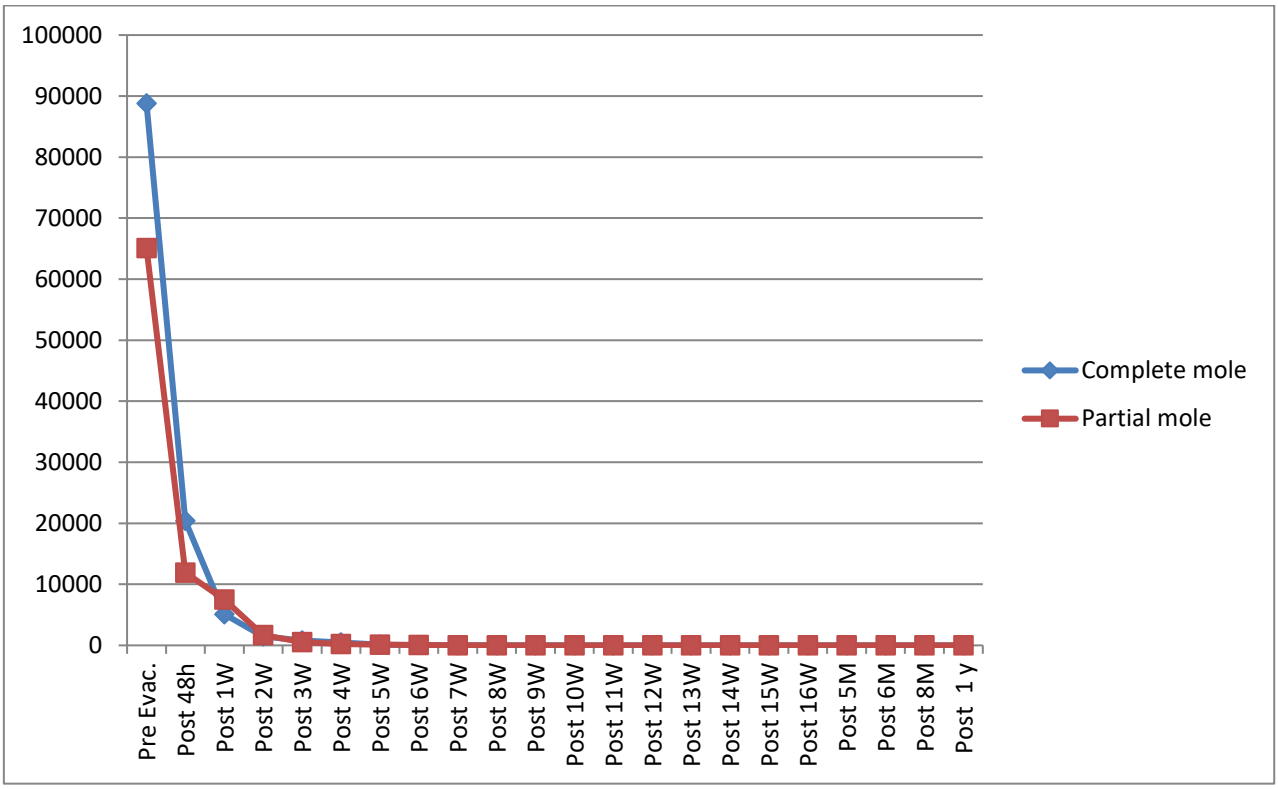
In table 4, complete and partial mole were compared regarding the demographic and clinical data; the age and existence of bilateral theca lutein cysts were significantly different between two types of moles (*P*= 0.045 & 0.024 respectively), there were no significant differences regarding pre-evacuation hCG (*P* = 0.29), and mean time to hCG normalization (*P* = 0.16).The rate of hCG decline after complete and partial molar evacuation was shown in figure 1.The percentage of cases which transformed to GTN is 24.2% in complete mole and 8% in partial mole (*P* = 0.105).

Table (4): Comparison between complete and partial mole regarding demographic and clinical data

Variables	**Complete mole (n=33) 57%**	**Partial mole (n=25) 43%**	**p-value**
Age/years	27.66±10.71	23.20±5.53	0.045
<18 18-<40 ≥40	7 (21.2%) 21 (63.6%) 5 (15.2%)	3 (12.0%) 21 (84.0%) 1(4.0%)	0.199
BMI	25.00±4.07	23.88±3.51	0.276
Parity Null& Primi para Multipara	 19 (57.6%) 14 (42.4%)	 13 (52.0%) 12 (48.0%)	0.672

Gest. Age (weeks)	8.75±2.30	9.72±3.12	0.183
Mode of delivery Caesarean Section Vaginal Delivery	 12 (54.5%) 10 (45.5%)	 12 (66.7%) 6 (33.3%)	0.436
History of abortion	11 (33.3%)	9 (36.0%)	0.832
Med. Disease	7 (29.2%)	8 (33.3%)	
Complaint Vaginal Bleeding Hyperemesis diagnosed by U/S PET Pelvic Pain	 9 (27.3%) 3 (9.1%) 21 (63.6%) 0 (0.0%) 0 (0.0%)	 11 (44.0%) 1 (4.0%) 11 (44.0%) 1 (4.0%) 1 (4.0%)	0.324
Theca Leutin Cysts			
Free	27 (81.8%)	25 (100%)	0.024
Bilateral and > 6 cm	6 (18.2%)	0 (0%)	
Blood transfusion need during evacuation	23 (69.7%)	12 (48.0%)	0.094
Mean pre-evacuation hCG (m.i.u/ml) Median (Min-Max) Mean ± SD	 88810 (3210-831000) 162970± 190737	 65109 (4300-780000) 107230± 161652	0.29
Mean time to hCG normalization (weeks) Median (Min-Max)	 8.5 (5-60) 11.37±10.8	 10 (5-16) 10.26±2.37	0.16
Progression to GTN	8/33 (24.2%)	2/25 (8%)	0.105

Figure (1): Comparison between complete and partial mole regarding different hCG levels before and after evacuation.



Fourteen GTN cases were reported; ten cases resulted progression of molar cases during follow up and four cases initially presented as GTN. All cases were low-risk (FIGO score 1-5). One case of invasive mole aged 42 years was treated with upfront hysterectomy with one course of methotrexate. Thirteen cases received 8 days regimen of intramuscular methotrexate-folinic acid; two of them failed to respond and were shifted to EMA/CO (etoposide, methotrexate, actinomycin D, cyclophosphamide and vincristine combination). One of these two cases who failed to combination chemotherapy underwent local myometrial resection followed by 2 courses of EMA/EP (etoposide, methotrexate, actinomycin D, etoposide, cisplatin) followed by hCG normalization.

Discussion

We reported for first time hospital -based and population – based incidence of molar pregnancy in Lower-Egypt population of 13.1 and 0.37 per 1000 live births respectively. Since data on the total number of pregnancies are not available; the denominator is live births which underestimate the population at risk which may result in a small overestimation of the incidence rates observed in the current study. The hospital-based incidence is more than reported in Taiwan (8.0 per 1000 deliveries), Indonesia (9.9 per 1000 pregnancies). Furthermore, our population based incidence is less than reported in the Netherlands (0.68 per 1000), Japan (3.0 per 1000) and England (1.54 per 1000) [19]. However, In Egypt and many developing countries spontaneous abortions specimens are not routinely subjected to histopathologic review and registration.

In this study, the mean age was 26.22±9.30 years with 71.0% of the cases between 18 and 40 years old which is quiet understandable as this is the child bearing age period for women with the maximum number of pregnancies which reported also by other authors [20-22]. The mode of delivery of the studied cases was; 57.1% caesarean deliveries and 42.9% vaginal deliveries which is compatible with the increasing trend in caesarean section rates in Egypt [23]. The percentage of the cases with previous history of abortion was 35.5%, a history of prior spontaneous abortion has been reported to give women a two to three-fold increase in molar pregnancy compared to a woman without such a history [24]. Anemia was the most common medical disease affecting 65.2% of the studied cases which goes with the previous studies concerning anemia among pregnant women in Egypt [25].

In this study we found that 53.2% of molar pregnancies were asymptomatic and accidentally discovered by ultrasonography which agreed with Joneborg et al [26] who reported that patients with vesicular mole were diagnosed before the onset of symptoms in 42.5% of cases, while Sun SY et al [27] reported that the most common presentation was vaginal bleeding in 46% compared with

33.9% in the current study. The sensitivity of ultrasound for accurately diagnosing hydatidiform mole was 87%, though Fowler et al and Kirk et al [28] reported that the sensitivity of ultrasound for accurately predicting hydatidiform mole was 44%. This discrepancy may be attributed to small sample size of our study.

The median level of hCG decline after molar evacuation for any type of GTD was 9 weeks which is earlier than reported by Delattre et al which was 12.3 weeks [29] which may explained by different patient criteria. Furthermore, we found that the GTN sequel during follow up was 10 cases (16.1%). Joneborg et al [30] reported that the risk of post-molar GTN was 8% in his study, though Schmitt et al [31] found that GTN developed in 12.1% of his cohort study. The variation of molar progression to GTN in different studies may reflect different outcome among different countries.

In literature a wide range of ratios of complete mole to partial mole incidence has been reported ranging from 0.3 to 3.0 [32]. In the present study; 33 complete mole case to 25 partial mole cases of total 58 cases with a ratio of 1.3. Morphologically, both complete mole and partial mole have distinct histopathological features; however, the subjective nature of the morphological characters may give rise to variation in diagnosis [33]. In particular when earlier evacuation is performed in the present ultrasound era, classic morphological features may be less distinct [34]. The differentiation between molar and non-molar gestations is usually clear in cases showing typical histological features, and in cases of complete mole this is confirmed by p57 immunohistochemistry [35]. However, the diagnosis of partial mole can still be confusing, even to specialized gynecological pathologists [36] and this is important clinically in view of the risk of GTN progression in these patients. Moreover, hydropic abortion has traditionally been considered the major differential diagnosis of partial mole [37].

The trend toward earlier diagnosis for both complete mole and partial mole observed in our center is consistent with the global trends as the median gestational age at evacuation was 8.75±2.30 weeks for complete mole and 9.72±3.12 weeks for partial mole. Sun et al reported The median gestational age at evacuation was 9 weeks for complete mole and 12 weeks for partial mole [38] and reported in another study [29] that the median gestational age at diagnosis continued to decrease in two non-concurrent cohorts (1988-1993 versus 1994-2013) of patients from the New England Trophoblastic Disease Center; 9 weeks versus 12 weeks. Blood transfusion was required during evacuation of 69.7% of cases of complete moles and 48.0% of partial moles. This figure is much more than reported by authors at the New England Trophoblastic Disease Center [39]. The high rate of blood transfusion in our study may be attributed to higher incidence of anemia in

Egyptian pregnant women which was reported by some authors [25] and correlated to high prevalence of anemia among the studied cases that was 65.2%.

The current study has limitations that Mansoura University Hospital is a regional referral center, and, therefore, our patient population may not be representative of the entire patient population. The reported data could be influenced by referral bias. Furthermore, immunohistochemical staining with P^{57} is not used routinely to differentiate between partial and early complete moles which may result in relatively higher incidence of partial moles. Centralization of GTD work allows proper estimation of incidence, sharing expertise at the national level, and help implementation of clear guidelines of diagnosis and treatment of GTD cases [40].

Conclusion

The incidence of molar pregnancy and GTN at Mansoura University Hospitals is estimated to be 13.1 and 3.2 per 1000 live births respectively. Ratio of complete to partial moles was 1.3. Fifty-three percent of the cases were accidentally discovered by ultrasound. We found no significance between CM and PM regarding mean pre-evacuation hCG level, mean time to hCG normalization, and rate of progression to GTN which need to be verified in future studies.

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Chapter 3

Outcome of Different Treatment Modalities for Gestational Trophoblastic Neoplasia in women aged 40 years and above: A Multicenter Retrospective Study

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The results of this study were presented during the ESGO 2019 meeting in Athens as "late breaking abstract".

Under Review

Abstract

Objective. To investigate the outcome of different treatment strategies in patients with gestational trophoblastic neoplasia (GTN) in women at 40 years old and above.

Methods. A multicentre study that retrospectively analysed data from 5 referral centres from 5 countries, including all women with GTN treated between 2012 and 2017, who were 40 years old and older. A total of 112 cases were eligible. Baseline characteristics and outcome of different treatment strategies were recorded and evaluated. The patients were categorized into low-risk non-metastatic, low-risk metastatic, and high-risk, based on the FIGO classification.

Results. Mean age of the included patients was 45.4 years ± 4.2SD. Of 80 patients with LR non-metastatic GTN, 46 women received single agent chemotherapy and 34 a hysterectomy with or without chemotherapy. Higher remission rate and shorter treatment duration (P=0.001) was seen in the group that underwent hysterectomy. Seven of the 14 patients with low-risk, metastatic GTN were cured with methotrexate. Two of the 18 high risk patients died before treatment, four were treated with polychemotherapy; two of them needed second line chemotherapy. Two cases received induction with methotrexate followed by EMA/CO. Ten high-risk patients were treated with hysterectomy and chemotherapy, of these; six achieved complete remission, three needed second line chemotherapy, and one patient died during chemotherapy treatment.

Conclusion. In this cohort; we found high proportions of metastatic and high risk cases, of methotrexate resistance, and of need for multiple treatment lines. In all groups hysterectomy was performed, but its role remains controversial in metastatic disease.

Key words: Gestational trophoblastic neoplasia, old age, treatment, outcome.

Introduction

The incidence of gestational trophoblastic neoplasia (GTN) increases with age; however, its treatment in women aged 40 years and above is poorly studied. Post-molar GTN after suction evacuation of molar pregnancies has been reported in 23 to 37% of women older than 40 years and 31 to 56% of women above 50 years of age [1]. In contrast, GTN following complete and partial moles have been reported in 20% and 5% of young women, respectively [2]. Moreover, Savage et al. reported a strong association of non-molar gestational choriocarcinoma with rising maternal age, rising from 1:162 917 for teenagers to 1:1197 for women aged over 45 [3]. In elderly women, complete moles are generally aneuploid, which is a risk factor for GTN [4].

To reduce this malignant sequel, prophylactic single-agent chemotherapy after molar evacuation has been recommended for "high-risk" moles in patients having maternal age above 40 years [5]. However, Jiang et al. [6] and Geng et al. [7] have reported no significant decrease in post-molar GTN after prophylactic chemotherapy. Primary hysterectomy reduces the tumour bulk and has been recommended in older patients with molar pregnancies to reduce the risk of developing GTN and need for chemotherapy [8, 9]. However, Giorgione et al. [10] reported that such treatment did not reduce the incidence of GTN. The International Federation of Obstetrics and Gynecology (FIGO) scoring system stratify patients with GTN to treatment decisions; one of the items included is the patient's age [11]. For the treatment of older GTN patients, it is debatable whether upfront hysterectomy should be performed to reduce tumour bulk or chemotherapy should be conducted without hysterectomy [12, 13]. Hysterectomy is recommended for placental-site trophoblastic tumours (PSTT) and epithelioid trophoblastic tumours (ETT) since both are often confined to the uterus and exhibit poor response to chemotherapy [14]. In the absence of international guidelines, treatment in most cases depends on nationwide guidelines, expert opinion, local hospital protocols, and the availability of resources in low-resource settings.

As of now, there are no established guidelines for the management of GTN at advanced age. Thus, we conducted this multicentre retrospective study to investigate the outcome of different treatment strategies in low-risk (LR) non-metastatic, LR metastatic, and high-risk (HR) GTN in patients aged 40 years or more.

Materials and methods

This study included all newly diagnosed patients with GTN from April, 2012 to March, 2017 in five referral centres (listed at the end of the paper). The Institutional Review Board of the faculty of Medicine, Mansoura University (Number.R.18.04.142), Mansoura, Egypt, gave central study approval.

Study population

The study included patients with GTN, i.e., invasive mole, choriocarcinoma, and post-molar GTN, and aged 40 years and older at the time of admission to any of the participating centres. Histopathological diagnosis was done by a team of specialized gynaecological pathologists in the participating centres. GTN cases were staged and classified according to the FIGO 2000 staging and classification guidelines [15]. Patients who refused initial permission to use their data for future research and patients whose histologic examination proved other pathologies rather than GTN were excluded. Since PSTT and ETT are rare and different tumour types that need a different approach, we decided to exclude these from this analysis.

Study design

From the patient charts, we retrieved age, parity, antecedent pregnancy, pre-treatment serum human Chorionic Gonadotropin (hCG) levels, presence of metastases, FIGO score, the need for single- and multi-agent chemotherapy, the number of courses necessary to achieve normalization of hCG concentrations, surgical procedures, histopathology, and outcome of treatment.

FIGO score < 7 is considered low-risk and ≥ 7 is considered high-risk. Since treatment rationale for metastatic versus non-metastatic disease might be different, we categorized the patients into three groups: LR non-metastatic, LR metastatic, and HR.

For each group, the following treatment modalities were recognized: single-agent chemotherapy with or without second curettage, polychemotherapy, and hysterectomy with or without adjuvant chemotherapy. Treatment outcome was accordingly evaluated. Resistance to chemotherapy was defined as plateauing or rising serum hCG for 3 consecutive weeks.

Disease recurrence was defined as a rise in serum hCG values after previous normalization, in the absence of a new pregnancy. Follow-up duration was considered to be at least 12 months after normalization of serum hCG.

Complications of chemotherapy were (in retrospect) graded according to the Common Terminology Criteria for Adverse Events (CTCAE, V. 3.0) [16].

Central study approval: Was obtained at Institutional Review Board (IRB) of Faculty of Medicine, Mansoura University, Egypt (IRB number: R.18.04.142).

Statistical methods.

Data were anonymized, imputed in an Excel database, and analysed using Statistical Package of Social Sciences (SPSS) software version 20.0 (IBM, Armonk, NY, USA). Qualitative data were described using numbers and percentages. Quantitative data were described using median (minimum, maximum and interquartile range) for non-parametric data. For parametric data, mean, standard deviation and 95% confidence interval (CI) were used. Normality was tested using Kolmogorov-Smirnov test. Significance of the obtained results was judged at 5% level, and all tests were two-tailed. Chi-square test was used for categorical variables to compare between different groups as appropriate. To compare parametric quantitative variables, the student's t test was used, and for non-parametric quantitative variables, the Mann-Whitney and Fischer exact tests were used. No core outcome sets were relevant for this study. Furthermore, there was no patient involvement in this retrospective study.

Results

Data of 141 patients from the participating centres were reviewed. Twenty-three cases were excluded: eight cases did not meet the time frame, seven cases had incomplete clinical data, two cases concerned a non-gestational choriocarcinoma, three cases were PSTT and three cases ETT. Finally, 112 cases with GTN who fulfilled the inclusion criteria were analysed (30 from Egypt, 13 from Ukraine, 14 from Canada, 30 from Saudi Arabia, and 25 from Indonesia). Complete details of the patients are given in the supplementary table. The mean age (years) was 45.4±4.2 SD (range; 40.0–55.0), and the median hCG at initial diagnosis of GTN was 2686 (range; 11–9609) IU/L. Ninety-four cases (83.9%) followed molar pregnancies, and 18 (16.1%) followed other pregnancy events. The commonest presentation was a raising hCG level after evacuation of a molar pregnancy, as it was observed in 63 cases (56.2%). LR non-metastatic GTN was observed in 80 (71.4%) cases, LR metastatic in 14 cases (12.5%) and HR in 18 cases (16.1%). FIGO stage I was observed in 50% cases. Metastases were diagnosed in 25 cases (22.3%), predominantly lungs (n = 20). Histopathological diagnoses were available for 76 cases either retrieved through second curettage (n = 14), a hysterectomy (n = 53), or after endometrial biopsy for abnormal uterine bleeding (n = 9). The commonest diagnosis was invasive mole (43.4%). The median follow-up duration was 17.5

months (range: 1–72 months). Seven cases (6.3%) were lost to follow-up within one year after normalization of serum hCG. Other sociodemographic data are shown in table 1.

Table 1. Demographic characteristics of the studied cases.

Age (years) (mean ± SD)	45.4 ± 4.2, range 40.0 - 55.0	
Parity median (range)	4 (0-12)	
Weight (Kg) (mean ± SD)	67.8 ± 17.8	
Serum HCG (IU/l) median (range)	2686 (11 - 9609)	
FIGO SCORE median (range)	2.7 (1-13)	
	Number N = 112	%
Antecedent pregnancy		
Unknown	5	4.5
Abortion	8	7.1
Complete mole	80	71.4
Partial mole	14	12.5
Term pregnancy	5	4.5
Histopathology	76	67.9
Specimen		
2nd curettage	14	18.4
hysterectomy	53	69.7
endometrial biopsy	9	11.8
Diagnosis		
Choriocarcinoma	23	30.3
Molar tissues	20	26.3
Invasive mole	33	43.4
Presentation [a]		
Raised hCG	63	56.2
Plateau hCG	8	7.1
Vaginal bleeding	55	49.1
Abdominal mass	4	3.6
Abdominal pain	4	3.6
Other	3	2.7
Metastasis.	N = 25	22.3
Site of metastases [b]		
Lung	20	80.0
Liver	1	4.0
Vagina	1	4.0
Renal	2	8.0
Urinary bladder	1	4.0
Fallopian tube	1	4.0
Bone	1	4.0
Treatment duration (days) median (range)	84.5(14-245)	
Follow up(months) median (range)	17.5(1-72)	
Outcome.		
Uneventful	97	86.6
Cured after relapse	5	4.9
Death	3	2.7
Lost follow up	7	6.3

[a] Presentation: Categories are not mutually exclusive. [b] Metastases: Some cases had more than one site.

Low-risk non-metastatic GTN.

The mean FIGO score in this subgroup was 3.2 (range, 1–6). Of the 80 patients with LR non-metastatic GTN, 46 patients received single-agent chemotherapy, in 14 preceded by a second curettage because of participation in a clinical trial [17]. Remission was achieved in 29 patients (63%). Sixteen (20%) patients needed second-line chemotherapy, one was cured with actinomycin-D as second-line single-agent therapy, 14 cases received polychemotherapy, and two underwent hysterectomy.

In 34 cases, upfront hysterectomy was performed. In four patients, a wait-and-see policy was successful. However, in most participating hospitals, the policy was to prescribe adjuvant chemotherapy, either a fixed number of courses (1 or 2) or based on regression of hCG levels. Therefore, instant chemotherapy followed in 30 patients, mainly single methotrexate with folic acid rescue (MTX/FA) (n = 29) for 1–12 courses. In 29 of 30 cases (96.7%), complete remission with uneventful follow-up was observed. In one patient, MTX/FA failed, but second-line etoposide was successful.

Compared to single-agent chemotherapy (with or without a second curettage), primary hysterectomy with or without single-agent chemotherapy was associated with a higher remission rate *(P*=0.001), a shorter mean treatment duration (*P*=0.001), and less number of total chemotherapy courses (mean 2.5 versus 5; $P < 0.001$) as shown in table 2

Table 2. Outcome of treatment of low-risk cases (n=80 cases).

	Monochemotherapy +/- second curettage (no=46)	Hysterectomy with or without chemo (n=34)	P value
Total chemotherapy Median (range)	5(1-20)	2.5(0.0-12.0)	z=3.48 p<0.001*
-Cured **-Needed 2nd or 3rd lines**	29(63.04) 17(36.96)	32(94.1%) 2(5.9%)	χ^2=10.42 p=0.001*
Relapse (n=79)	1(2.2)	0 (0)	FET P=1.0
Treatment duration	92(16-245)	45.0(14-154)	z=3.18 p=0.001*

z: Mann Whitney U test χ^2:Chi-Square test

FET :Fischer exact test.

Low-risk, metastatic GTN.

The mean FIGO score in this subgroup was 4.0 (range 2 – 6). Eight of the 14 women with LR metastatic GTN were initially treated with MTX/FA. Four of them reached complete remission, and four had incomplete responses. Of these four patients, one was cured after second-line single-agent actinomycin-D, one successfully received second-line polychemotherapy, one received second-line polychemotherapy combined with hysterectomy, and one received 7 courses of etoposide,

methotrexate, actinomycin-D, cyclophosphamide and vincristine polychemotherapy (EMA/CO) and underwent excision of vaginal metastases. In this patient, a relapse that occurred 2 years later was cured with EMA/CO.

In six patients, a hysterectomy was performed, followed immediately by MTX/FA. This resulted in complete response with uneventful follow-up in three patients. Two were cured with second-line chemotherapy EMA/CO, and the third failed on second-line single carboplatin but achieved complete remission with third-line EMA/CO. This patient had a pulmonary relapse after 7 months, which was again treated with EMA/CO. Only 7 of the 14 patients with LR metastatic GTN were cured with MTX/FA chemotherapy, 3 of whom also underwent a hysterectomy. All others needed second- or third-line chemotherapy, and two developed a relapse.

High-risk GTN

The mean FIGO score in this subgroup was 8.3 (range, 7–13). Two high-risk patients died before treatment could start due to distant metastases. In six patients, chemotherapy was started; two cases were started with induction MTX/FA due to bad general condition, followed by EMA/CO. Relapse occurred in one patient who was treated again with EMA/CO. In four patients, treatment started with EMA/CO chemotherapy; two of them had incomplete response and subsequently received second-line etoposide, MTX/FA, actinomycin-D, etoposide, and cisplatin (EMA/EP).

Ten cases (62.5%) underwent hysterectomy and mono or poly chemotherapy as first-line. One patient died during chemotherapy treatment, six (60%) showed complete response, one of them developed renal metastases, which was successfully treated with nephrectomy and EMA/CO, and three (30%) needed second-line treatment to achieve a complete response.

The complications of chemotherapy could be retrieved for 42 cases (37.5%) only. Grade 3 complications were reported in 4 cases (9.5%) including renal failure, respiratory failure, deep venous thrombosis, and severe anaemia.

Discussion

We presented the experience of five different centres in five countries on four continents (Africa, Asia, Europe, and North America) in managing GTN in patients aged 40 years and above. In the current study, 44% of cases with LR non-metastatic GTN did not achieve remission with initial treatment with MTX/FA, which is higher than the expected 10% in all age categories [18]. In an analysis of 359 patients with low-risk GTN treated between 1979 and 2006 at the Brewer Trophoblastic Center (Chicago), approximately 80% of women were cured with first-line single-

agent therapy, mainly MTX/FA [19]. In our study, 11 of 14 cases (69.6%) with LR non-metastatic disease achieved complete remission after second curettage plus MTX/FA. Most of these cases were included in a randomized study conducted by Hemida et al. [17]. Overall, this study did not find a significant reduction in the number of chemotherapy courses among patients who underwent second curettage and who did not. The study did not stratify for age, and a subgroup analyses for older aged patient was not performed. Thus, we would recommend hysterectomy over second curettage. For LR metastatic disease, complete remission was observed only in 50% of cases with single-agent chemotherapy with or without hysterectomy. Goldstein et al. [1] reported that patients with low-risk metastatic GTN (Stages II and III, score < 7) were treated with single-agent chemotherapy with cure rates of 80–90% for all ages. Our result is, however, in line with two cohort studies: Dutch [20] and British [21] studies that reported higher MTX resistance and recurrence rates in patients with lung metastases than in patients without lung metastases. This may denote a poorer response to single-agent chemotherapy regardless of age and would plead for larger, preferably prospective, cohort studies.

A higher acceptance of hysterectomy is observed in the older age group when compared to the younger group with a wish to preserve fertility. Hysterectomy was performed in 43% LR cases, regardless of the presence or absence of metastases. This resulted in a remission rate of 97% in the 34 non-metastatic LR GTN, opposed to 50% in the six LR metastatic disease. This result is in line with the findings of Bolze et al., [22] who reported a retrospective analysis on 74 patients who underwent hysterectomy as first-line treatment; 82% of them did not require any further salvage chemotherapy. These results are better than the findings of Eysbouts et al., [23] who reported complete remission after hysterectomy in 47.8% patients. The high cure rate after hysterectomy in our LR non-metastatic group may be attributed to the prophylactic use of 1–2 courses of MTX/FA with hysterectomy in most participating centres in this study. Rodriguez et al. [24] also supported this concept. The role of hysterectomy in the LR metastatic group is less clear; Eysbouts et al. reported that no cases with metastatic GTN achieved remission with hysterectomy alone [23]. In our study, hysterectomy was often chosen at the start of treatment, regardless of the low- or high-risk status and presence of metastasis. Hysterectomy is thought to reduce the burden of disease and, therefore, improve outcome in terms of reduction of the number of chemotherapy courses and less failure of first-line chemotherapy. It remains debatable whether this applies to patients with metastatic disease as surgery should not delay the prompt start of chemotherapy since it is the cornerstone of treatment in metastatic disease.

In the current study, 15% were categorized as high-risk GTN, which is similar to the results reported by other authors who reported 10% incidence of HR GTN in all age groups [18]. Ten of 18

(55.6%) HR patients were initially treated with hysterectomy with chemotherapy in contrast to only four patients (22.2%) who started with combination chemotherapy alone. This indicates the more frequent use of hysterectomy in this age group. Adjuvant hysterectomy is likely to benefit cautiously selected patients with high-risk GTN [25, 26], but polychemotherapy is the standard of care. Fear for deterioration of the clinical condition in cases with a high tumour burden was at stake in some cases, resulting in the adaptation of the chemotherapy schedule. In our study, two cases of HR received MTX/FA induction prior to EMA/CO. Alifrangis et al. [27] used etoposide-cisplatin induction chemotherapy before EMA/CO in 23% of high-risk patients with a large disease burden.

We reported a relapse rate of 4.6%, which is in agreement with that reported by other authors [26]. Three fatal cases were reported in the current study (2.7%), which is twice the mortality rate reported by Ozlap et al. [18] in a retrospective study involving GTN cases in all age groups in Turkey. However, when we compare our results with the published data; the groups may not be directly compatible.

Our study demonstrates that the majority of the participating centres use nationwide or local hospital protocols in the absence of international guidelines for the treatment of GTN at 40 years of age and above. Thus, there is an urgent demand for international guidelines [21].

The limitations of the current study include its retrospective design, missing data (i.e., chemotherapy toxicity was retrieved for only 37% of patients from the charts), heterogeneity of data as reported by different centres, and different local hospital protocols for management. Unfortunately, the central pathology review of histopathology could not be obtained, but all cases were treated in a national or regional center, where experienced pathologist examined the available specimens. Uniform international definition and treatment would make it easier to combine datasets from different global cohorts and address certain topics currently unanswered in the community [21].

The strength of the study is that it reflects a relatively large group of patients from tertiary centers across the world. It, therefore, reflects practice as performed under different circumstances. The unexplained high number of hysterectomies in LR metastatic and HR disease is of concern since this might delay the start of (poly) chemotherapy and impact outcome.

Conclusion

GTN at 40 years old or above may have a poorer prognosis than women of younger age, with a larger proportion of metastatic, high-risk, and MTX resistant disease. Hysterectomy with MTX/FA for LR non-metastatic disease was successful with a remission rate above 90%. Hysterectomy was also widely used in metastatic LR and HR disease, but its role remains controversial in these groups and

chemotherapy should not be delayed because of the surgery. A large, randomized study is needed to investigate the optimal treatment strategy.

List of Abbreviations.

GTN: gestational trophoblastic neoplasia. LR: low-risk. HR: high-risk. hCG: human chorionic gonadotropin. MTX/FA: methotrexate/folinic acid. EMA/CO: etoposide, methotrexate, actinomycin-D, cyclophosphamide and vincristine. IU/L: international unit per litre.

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HvD: study design and manuscript editing.

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Legends to tables.

- Table 1. Demographic characteristics of the studied case

-Table 2. Outcome of treatment of low-risk cases (n=80 cases).

- Appendix 1. Supplementary file; case details.

Appendix 1: Supplementary file; case details

I- Low risk cases-non metastatic. (n = 80)

A. Single-agent chemotherapy (n = 32)

Complete response (n = 18)

Uneventful follow up (FU) (n = 16)

Case 5, received 3 courses of Methotrexate (MTX), FU 20 months.
Case 44, received 3 courses of MTX, FU 22 months.
Case 43, received 4 courses of MTX, FU 25 months.
Case 67, received 4 courses of MTX, FU 47 months.
Case 81, received 4 courses of MTX, FU 12 months.
Case 4, received 5 courses of MTX, FU 23 months.
Case 101, received 5 courses of MTX, FU 24 months.
Case 71, received 6 courses of MTX, FU 12 months.
Case 50, received 6 courses of MTX, FU 20 months.
Case 59, received 7 courses of MTX, FU 24 months.
Case 69, received 7 courses of MTX, FU 24 months.
Case 58, received 8 courses of MTX, FU 15 months.
Case 86, received 8 courses of MTX, FU 12 months.
Case 57, received 9 courses of MTX, FU 29 months.
Case 47, received 9 courses of MTX, FU 45 months.
Case 52, received 13 courses of MTX, FU 17 months.

Relapse (n = 1)

EMA/CO for relapse after complete response

Case 25, received 5 courses of MTX, complete response. Relapse, treated with 4 courses of EMA/CO, FU 16 months.

Lost to FU (n = 1)
Case 36, received 4 courses of MTX, FU 6 months.

Incomplete response (n = 14)

2nd line polychemotherapy (n = 12)

Complete response to 2nd line chemotherapy, further FU (n = 12)

Case 110, failed to 2 MTX, received 3 EMA/CO, FU 36 months.
Case 74, failed to 5 MTX, received 3 EMA/CO, FU 20 months.
Case 72, failed to 15 courses of MTX, received 5 courses of EMA/CO, FU 15 months.
Case 34, failed to MTX, received 1 course of 2nd line chemo, FU 72 months.
Case 22, failed to MTX, received 2 courses of EMA/CO, FU 12 months.
Case 35, failed to MTX, received 2 courses of 2nd line chemo, FU 60 months.
Case 65, failed to MTX, received 2 courses of EMA/CO, FU 27 months.
Case 29, failed to MTX, received 3 courses of EMA/CO, FU 20 months.
Case 70, failed to MTX, received 3 courses of EMA/CO, FU 12 months.

Case 9, failed to MTX, received 4 courses of EMA/CO, FU 24 months.
Case 33, failed to MTX, received 6 courses of EMA, FU 48 months.
Case 26, failed to MTX, received 8 courses of EMA/CO, FU 17 months.

further hysterectomy (n = 2)

Case 40, failed to MTX, 2nd line hysterectomy. Pathology; choriocarcinoma, FU 17 months.

Case 41, failed to MTX, 2nd line hysterectomy. Pathology; complete mole, FU 13 months.

B. Curettage and chemotherapy (n=14)

Complete response (n = 11)

Uneventful FU (n = 9)
Case 96, received 1 course of MTX, FU 56 months.
Case 98, received 1 course of MTX, FU 12 months.
Case 105, received 1 course of MTX, FU 23 months.
Case 106, received 1 course of MTX, FU 12 months.
Case 107, received 1 course of MTX, FU 12 months.
Case 2, received 2 courses of MTX, FU 20 months.
Case 7, received 2 courses of MTX, FU 12 months.
Case 8, received 4 courses of MTX, FU 12 months.
Case 24, received 4 courses of MTX, FU 12 months.

Lost to FU (n = 2)
Case 103, received 1 course of MTX, FU 1 month.

Case 90, received 3 courses of MTX, no FU.

Incomplete response: (n = 3)

Complete response to 2nd line polychemotherapy, further FU (n = 2)

Case 3, failed to MTX, received 4 courses of EMA/CO, FU 20 months.

Case 6, failed to MTX, received 5 courses of EMA/CO, FU 12 months.

Complete response to 2nd line polychemotherapy, lost to FU (n = 1)

Case 91, failed to MTX, received 3 courses of EMA/CO, FU 6 months.

C. Hysterectomy (n = 4)

Complete response, further FU (n = 4)

Case 38, pathology; invasive mole, FU 12 months.

Case 46, pathology; invasive mole, FU 18 months.

Case 84, pathology; invasive mole, FU 14 months.

Case 104, pathology; invasive mole, FU 12 months.

D. Hysterectomy and chemotherapy* (n = 30)

* One case (case 10) EMA/CO, all others MTX

Complete response (n = 29)

Complete response, uneventful FU (n = 28)

Case 19, pathology; invasive mole, received 1 course of MTX, FU 12 months.
Case 23, pathology; invasive mole, received 1 course of MTX, FU 12 months.
Case 94, pathology; choriocarcinoma, received 1 course of MTX, FU 12 months.
Case 111, pathology; complete mole, received 1 course of MTX, FU 12 months.
Case 10, pathology; choriocarcinoma, received 2 courses of EMA/CO, FU 36 months.
Case 13, pathology; invasive mole, received 2 courses of MTX, FU 18 months.
Case 14, pathology; invasive mole, received 2 courses of MTX, FU 42 months.
Case 15, pathology; invasive mole, received 2 courses of MTX, FU 36 months.
Case 16, pathology; invasive mole, received 2 courses of MTX, FU 30 months.
Case 17, pathology; choriocarcinoma, received 2 courses of MTX, FU 14 months.
Case 21, pathology; invasive mole, received 2 courses of MTX, FU 12 months.
Case 32, pathology; invasive mole, received 2 courses of MTX, FU 48 months.
Case 12, pathology; invasive mole, received 3 courses of MTX, FU 18 months.
Case 18, pathology; invasive mole, received 3 courses of MTX, FU 23 months.
Case 63, pathology; invasive mole, received 3 courses of MTX, FU 19 months.
Case 76, pathology; invasive mole, received 3 courses of MTX, FU 12 months.
Case 85, pathology; invasive mole, received 3 courses of MTX, FU 12 months.
Case 20, pathology; invasive mole, received 4 courses of MTX, FU 14 months.
Case 75, pathology; invasive mole, received 4 courses of MTX, FU 58 months.
Case 77, pathology; choriocarcinoma, received 4 courses of MTX, FU 31 months.
Case 61, pathology; choriocarcinoma, received 5 courses of MTX, FU 60 months.
Case 73, pathology; invasive mole, received 5 courses of MTX, FU 12 months.
Case 83, pathology; invasive mole, received 6 courses of MTX, FU 25 months.
Case 28, pathology; complete mole, received 6 courses of MTX, FU 14 months.
Case 31, pathology; invasive mole, received 6 courses of MTX, FU 24 months.
Case 88, pathology; invasive mole, received 7courses of MTX, FU 12 months.
Case 60, pathology; invasive mole, received 8 courses of MTX, FU 24 months.
Case 48, pathology; complete mole, received 12 courses of MTX, FU 16 months.

Complete response, lost FU (n=1):

Case 95, pathology; invasive mole, received 1 course of MTX, FU 6 months.

Incomplete response (n =1)

2nd line chemotherapy after hysterectomy (n = 1)

Case 54, Pathology invasive mole, failed to MTX (7 courses), 2nd line 3 courses of etoposide, FU 17 months.

II-Low risk cases - metastatic (n = 14)

A. Hysterectomy and single agent chemotherapy* (n = 6).

* In all cases MTX

Complete response: (n = 3)

Case 97, pathology; choriocarcinoma, received 1 course of MTX, FU 43 months.

Case 79, pathology; invasive mole, received 3 courses of MTX, FU 19 months.

Case 78, pathology; invasive mole, received 5 courses of MTX, FU 17 months.

Incomplete response (n = 3)

polychemotherapy after hysterectomy and single-agent chemotherapy (n = 3)

complete response: (n = 2)

Case 66, pathology; choriocarcinoma. Failed to MTX(5 courses). 2nd line 5 courses of polychemotherapy, FU 27 months.

Case 68, pathology; invasive mole. Failed to MTX (6 courses). 2nd line 2 courses of polychemotherapy, FU 33 months.

Incomplete response (n = 1) 2nd and 3rd line chemotherapy, relapse and 4th line

Case 37, pathology; choriocarcinoma. Failed to MTX(4 courses), 2nd line failed (2 courses of carboplatin), 3rd line 3 courses of EMA/CO. Complete response. Lung relapse after 7 months, treated with EMA/CO and cured, FU 17 months.

B. Single-agent chemotherapy (n = 8)

Complete response

Uneventful FU: (n = 4)

Case 62, received 3 courses of MTX, FU 24 months.

Case 80, received 3 courses of MTX, FU 15 months.

Case 109, received 5 courses of MTX, FU 12 months.

Case 64, received 9 courses of MTX, FU 22 months.

Incomplete response (n = 4)

2nd line actinomycine D after MTX (n = 1)

Case 49, failed to MTX (4 courses), 2nd line received 4 courses of actinomycine -D, FU 17 months.

2nd polychemotherapy after MTX (n= 1)

Case 27; failed to MTX (2 courses), 2nd line received 7 courses of EMA/CO, FU 12 months.

2nd line polychemotherapy and 3rd line surgery (n = 2)

Uneventful after surgery

Case 82, failed to MTX (7 courses), 2nd line failed (2 courses of polychemotherapy), 3rd line hysterectomy. Pathology; invasive mole, FU 13 months.

Relapse after surgery

Case 108, failed to MTX(1 course), 2nd line failed (7 courses of polychemotherapy), 3rd line excision of vaginal metastasis. Pathology: choriocarcinoma. Relapse after 2 years, treated with EMA/CO, FU 24 months.

III-High risk patients (n = 18)

A. Death prior to treatment start (n = 2)

Case 93, died before start of treatment.

Case 112, died before start of treatment.

B. Hysterectomy and chemotherapy (n= 10)

Died during treatment (no=1)

Case 100, pathology; choriocarcinoma, received 5 courses of EMA/CO, Died during treatment.

Complete response (n = 7)

Uneventful FU (n = 5)

Case 11, pathology; choriocarcinoma, received EMA/CO, FU 40 months.

Case 45, pathology; choriocarcinoma, received 6 courses of EMA/CO, FU 63 months.

Case 55, pathology; Invasive mole, received 1 course of EMA/CO, FU 18 months.

Case 87, pathology; invasive mole, received 6 courses of EMA/CO, FU 15 months.

Case 102, pathology; invasive mole, received EMA/CO, FU 12 months.

Lost to FU (n = 1)

Case 39. pathology; choriocarcinoma, received EMA/CO, FU 10 months.

Relapse (n = 1)

Case 30, pathology; choriocarcinoma, received EMA/CO, complete response. Relapse (renal), treated with nephrectomy and 3 courses of EMA/CO, FU 36 months.

Incomplete response, further treatment polychemotherapy (n = 2)

2nd line polychemotherapy (n = 2)

Complete response to 2nd line polychemotherapy, further FU (n = 1)

Case 42, failed to MTX (5 courses) 2nd line 2 courses of EMA/CO. FU 12 months.

Complete response to 2nd line polychemotherapy, lost FU (n = 1)

Case 99, hysterectomy; choriocarcinoma, failed to EMA/CO (5 courses), received 5 courses of 2nd line chemotherapy with complete response, FU 10 months.

C. Induction monochemotherapy followed by polychemotherapy (n = 2)

(because of poor general condition)

Complete response (n = 2)

Uneventful FU(n =1)

Case 89, received 1 course of MTX followed by 5 courses of EMA/CO, FU 44 months.

Relapse (n =1)

Case 92, received 1 course of MTX followed by 5 courses of EMA/CO. Relapse treated with EMA/CO, FU 43 months.

D. Poly chemotherapy (n = 4)

Complete response (n = 2)

Uneventful FU (n = 2)

Case 53, received 7 courses of EMA/CO, FU 17 months.

Case 51, received 9 courses of EMA/CO, FU 27 months.

Incomplete response (n = 2)

2nd line polychemotherapy (n = 2)

Complete response to 2nd line chemotherapy, further FU (n = 2)

Case 56, received 7 courses of EMA/CO, incomplete response, 2nd line 5 EMA/EP, FU 24 months.

Case 113, received 5 courses of EMA/CO, incomplete response, 2nd line EMA/EP, FU 12 months.

Chapter 4

The Impact of Second Uterine Curettage on the Number of Chemotherapy Courses in Low-risk Postmolar Gestational Trophoblastic Neoplasia. A Single-Centre, Randomized Controlled Study

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Abstract

Background

The value of second uterine curettage in postmolar gestational trophoblastic neoplasia (GTN) is presently unclear. In a randomized study of low-risk postmolar GTN, we investigated the effect of second uterine curettage on the number of chemotherapy courses and relapse rate, and on variables associated with the number of chemotherapy courses.

Methods

Eligibility criteria were age 18 or over, WHO low or intermediate risk score for postmolar GTN, serum β human chorionic gonadotropin (hCG) level ≤ 5,000 IU / L, and fit for treatment with methotrexate. Exclusion criteria were previous uterine perforation and/or life-threatening bleeding. Randomization was performed (1:1) to a second curettage (intervention) or no curettage (control) group before methotrexate treatment, stratified for hCG level and vaginal bleeding. The primary outcome was the number of chemotherapy courses required for hCG normalization. Secondary outcomes were need for second-line treatment, toxicity, relapse rates, and variables associated with number of chemotherapy courses. The study was registered in the Dutch Trial Registry (NTR3390).

Results

Eighty-six consecutive patients presenting with postmolar GTN were included in the intention to treat analyses. Groups were comparable with regard to demographics and patient characteristics. Surgical complications did not occur. The mean number of chemotherapy courses required to reach hCG normalisation was 4.4 ± 2.2 (standard deviation (SD)) in the control group versus 3.8 ± 2.3 SD in the intervention group ($p = 0.14$). Groups were comparable in terms of second-line treatment needed to reach hCG normalisation (3 and 4 cases, respectively), and relapse within the first year (2 and 1 cases, respectively).

Conclusion

Second uterine curettage has no impact on the number of chemotherapy courses required or on the rate of relapse in low-risk postmolar GTN patients.

Key words:

Gestational trophoblastic neoplasia; treatment; Second uterine curettage.

Introduction.

Gestational trophoblastic neoplasia (GTN) includes postmolar GTN [1], which is typically diagnosed in asymptomatic women undergoing routine serum hCG monitoring after evacuation of a complete or partial molar pregnancy [2]. Following diagnosis of postmolar GTN, staging and risk assessment (mostly using FIGO staging and the modified World Health Organisation (WHO) Prognostic Scoring System as adapted by FIGO)[3] distinguishes patients at low versus high-risk, which influences subsequent decisions on the specific chemotherapy regimen. At present, most low-risk (WHO risk score 0–6) GTN patients are treated with single-agent methotrexate or actinomycin D [4], and remission rates of 90% and over are obtained in stage I disease, compared to rates of approximately 70% in stage II and stage III disease[4,5].

The role of second uterine curettage, as a single or additional treatment, in the management of postmolar GTN is unclear. Previous retrospective studies found widely differing cure rates, varying between 9 and 80% [6,7]. A debulking effect of second uterine curettage has been shown in two retrospective analyses which reported that fewer chemotherapy courses were needed to reach undetectable serum hCG levels after second curettage[6,8]. This reduction seems to be related to serum hCG level and the presence or absence of myometrial invasion and distant metastases.

Recently, two prospective observational studies were published, including a small prospective pilot study which reported cure in ten out of twelve patients after second curettage in postmolar GTN [9]. The second, a study performed by the Gynecologic Oncology Group, reported cure rates of 40% for low-risk non-metastatic GTN using second uterine curettage as a single treatment [10]. The disadvantages of second uterine curettage include complications such as uterine perforation, infection and bleeding, and a delay in starting chemotherapy when post-curettage hCG levels fail to normalise [10].

In a single-centre, randomized phase II trial in low-risk postmolar GTN patients, we investigated the effect of second uterine curettage on the number of chemotherapy courses needed to achieve hCG normalisation, and on the toxicity and relapse rates. We also aimed to identify variables associated with the number of courses required for successful treatment.

Methods

Study design and participants

In this randomised, controlled phase II trial, carried out in a single university centre in Mansoura, Egypt, consecutive patients with low-risk postmolar GTN and serum hCG levels ≤ 5,000 IU/L were

considered eligible. The Mansoura University Hospital provides tertiary healthcare for most of the Delta region of Egypt, with a population of about 12 million. Most of the required treatments (second uterine curettage, methotrexate and folinic acid) were provided free of charge to reduce the cost burden on patients and to minimize drop-out.

Eligible participants were women aged 18 or over with a postmolar GTN, with a WHO low or intermediate risk score, a serum hCG level ≤ 5,000 IU/L, and fit for standard treatment with methotrexate. Exclusion criteria were previous uterine perforation and/or life-threatening bleeding. Patients gave written informed consent before inclusion in the study. The study was approved by the ethical committee of the Faculty of Medicine, Mansoura University (Number R/48) and was registered in the Dutch Trial Registry (NTR3390).

Randomization

Patients were randomized (1:1) by means of a web-based system, using random block sizes, at the Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands. The patients were stratified according to the presence or absence of vaginal bleeding and the level of baseline serum hCG, i.e., < 1,500 IU/L or 1,500-5,000 IU/L.

Procedures

At baseline, all patients underwent a pelvic transvaginal ultrasound examination to measure the dimensions of intra-uterine disease (mm^2), calculated as the product of the longest versus the perpendicular diameter of the uterine lesion. In addition, patients underwent a staging chest X-ray and hCG measurement (assay used not specified, normal value < 7 IU/L). Central pathology review of the primary curettage was not performed.

Second uterine curettage was performed under short-acting general anaesthesia or sedation, and antibiotic prophylaxis. After cervical dilatation the uterine cavity was evacuated using manual vacuum aspiration under ultrasound guidance (Ipas MVA EasyGrip® Cannulae, Ipas, Chapel Hill, NC 27515 USA) [11,12]. Tissue obtained was sent for histopathological analyses.

All patients received standard methotrexate treatment with folinic acid support, both given intramuscularly in a 2-weekly regimen. Methotrexate was administered at a dose of 50 or 75 mg (the latter for women exceeding 75 kg) on days 1, 3, 5 and 7, alternating with folinic acid at a dose of 12.5 mg on days 2, 4, 6 and 8. The first methotrexate course was given during admission, and subsequent courses were given at the woman's home by a trained nurse. After normalisation of serum hCG, two consolidation courses of methotrexate were given.

Before each course of chemotherapy, a complete blood cell count, kidney and liver function tests, and serum hCG were evaluated. Adverse events were defined and graded using Common Terminology Criteria for Adverse Events (CTCAE) v3.0 [13]. In patients who experienced severe toxicity or failure of methotrexate, treatment was abandoned and further management was given according to tumour board recommendations. Methotrexate failure was deemed to be a plateau or decline of less than 10% in hCG levels over 3 courses, or a rise in the hCG level of more than 10% above the prior obtained value over two consecutive courses. In case of methotrexate failure or relapsed GTN, re-staging was performed. The choice of second-line chemotherapy was individualized according to disease status, tumour board recommendations and drug availability. During and after treatment, patients were strictly advised not to become pregnant. Follow-up was performed by monthly testing of serum hCG levels for a year after normalization of hCG.

Primary and secondary outcome measures

The primary endpoint was the mean number of chemotherapy courses needed to achieve hCG normalisation. Secondary endpoints were "successful treatment", defined as normalization of serum hCG level by methotrexate only and without relapse within one year. "Second-line treatment" was defined as treatment after methotrexate failure, the need for emergency surgery and any treatment for relapse during follow-up. Other secondary endpoints were length of chemotherapy cycles, complications and toxicity of methotrexate, the need for surgery, and variables associated with the number of methotrexate courses until hCG normalisation.

Statistical analyses

Based on previously published data, we expected to find a mean reduction from 4.8 chemotherapy courses to 2.5 courses before hCG normalization [6,8]. With a two-sided 5% significance level, a power of 99%, and an expected dropout rate of 10%, a sample size of 44 patients per group was necessary to detect this difference. We anticipated that a 36-month inclusion period would be required to recruit this number of patients.

Analyses were performed as intention-to-treat. Sociodemographic characteristics and treatment outcomes between the two arms were compared using the Mann-Whitney U test for continuous variables and the Kruskal-Wallis test for categorical variables. Linear regression was performed to study the effect of second uterine curettage and other variables on the number of chemotherapy courses needed. A negative odds ratio (OR) means the variable was associated with fewer chemotherapy courses. To study the effect of several variables on treatment success, which is a dichotomous outcome, logistic regression was performed. An OR below one means the variable

was associated with less frequent treatment success. Multivariable regression analysis was performed with all relevant variables, with a maximum degree of freedom of 1 per 10 patients. Statistical tests were two-sided and p-values were considered statistically significant when $p < 0.05$. Statistical analyses were carried out using the statistical package SPSS 22 for Windows (SPSS, Chicago, IL, USA).

Results

Between October 2011 and February 2016, 123 patients with postmolar GTN were seen at the Mansoura Trophoblastic Clinic, of whom 34 were excluded for the following reasons; 26 patients did not fulfil the eligibility criteria, five eligible patients refused randomization, two started treatment in another hospital, and one needed immediate emergency surgery for severe uterine bleeding. Consequently, 89 consecutive patients were enrolled in the study. After randomization three patients were excluded; one chose hysterectomy in another hospital, the second refused subsequent treatment when methotrexate failed and was lost to follow-up, and the third ultimately did not fulfil the diagnostic criteria of postmolar GTN. Therefore, 86 patients were finally included in the intention-to-treat analyses (figure 1).

Patient groups were comparable for age, parity, hCG level, type of preceding mole, size of uterine mass, WHO performance score, weight and BMI (table 1).

Table 1. Sociodemographic data

	Methotrexate N = 43	**Second curettage and methotrexate N = 43**	**P value**
Age in years Mean(SD)	26.6 (6.4)	25.8 (7.7)	0.63
Parity			
• Nulliparous	12	10	0.62
• Parous	31	33	
Preceding pregnancy			
• Complete mole	29	27	0.65
• Partial mole	14	16	
hCG pattern			
• Rising	27	30	0.63
• Plateau	13	12	
• Persistent	3	1	
Vaginal bleeding			
• Vaginal bleeding present	28	23	0.28
• No vaginal bleeding	15	20	

WHO score • 0 • 1 • 2	 1 33 9	 0 32 11	0.49
Uterine mass (mm^2) Mean(SD)	139 (19)	171 (27)	0.46
βhCG level (IU/L)	3000 (1974)	3274 (1813)	0.59
Weight	75.5 (12.4)	71.5 (10.5)	0.09
BMI	26.9 (3.3)	26.5 (3.0)	0.46

Table 2. Treatment outcome

	Methotrexate (n = 43)	**Second curettage and methotrexate (n = 43)**	**P value**
Mean number of courses to reach normalization of βhCG (SD)(*)	4.4 (2.2)	3.8 (2.3)	0.14
Mean duration of single cycle (days) (SD)	17.9 (4.1)	17.9 (4.6)	0.58
Complications of methotrexate (^)			0.57
• None	30(69.8%)	28(65.1%)	
• at least one complication	13 (30.2%)	15(34.9%)	
▪ Grade 1			
Pharyngitis	9 (20.95)	6 (14.0%)	
Colic pains	3 (7.0%)	3 (7.0%)	
▪ Grade 2			
Fatigue	15 (34.9%)	13 (30.2%)	
Oral Ulcers	8 (18.6%)	7 (16.3%)	
Vaginal bleeding, requiring intervention	1 (2.3%)		
▪ Grade 3			
Vaginal bleeding requiring surgery (°)	2 (4.7%)		
Treatment outcome			0.77
• Successful	36 (83.7%)	37 (86.0%)	
• Combination chemotherapy after methotrexate failure	3 (6.9%) 2 (4.6%)	4 (9.3%)	
• Need for emergency intervention			
• Relapse < 1 year	2 (4.6%)	1 (2.3%)	
• Choriocarcinoma after subsequent pregnancy		1[$] (2.3%)	
Histology[#]			
• Molar tissue	2 (4.6%)	35 (83.3%)	
• Nonmolar tissue		7 (16.7%)	
• Choriocarcinoma		1[$] (2.3%)	
Follow -up			0.96
• Duration (months)	23 (14)	23 (13)	
• Lost to follow-up	2	1	
• Pregnancy within 12 months after normalization of βhCG	7	6	

(*) Sum of methotrexate and second-line treatment in the first episode.

(^) Grades of complications according to CTCAE criteria[13]. A patient can suffer from more than one complication.

(°) Emergency curettage (n = 1) and emergency hysterectomy (n = 1).

($) Diagnosis of invasive choriocarcinoma shortly after delivery of the subsequent pregnancy.

(#) In the standard methotrexate group, histology was obtained twice; once by emergency curettage for bleeding, and once by emergency hysterectomy after methotrexate failure. In the intervention group, histology was missing for one curettage. The choriocarcinoma was diagnosed after a subsequent pregnancy in a case where the second curettage showed no molar tissue.

The mean number of chemotherapy courses required for hCG normalization was comparable for the control group, 4.4 ± 2.2 SD, and the intervention group, 3.8 ± 2.3 SD (p = 0.14) (table 2). Mean cycle duration was 17.9 days in both groups. Results of the unforeseen substandard care analyses and details on unsuccessful cases are described in supplementary table 1. Methotrexate failure was similar for both groups; in three control cases and four intervention cases, respectively. During the first year of follow-up, disease relapse occurred in two patients in the control arm and in one patient in the intervention group, also in the intervention group one patient was diagnosed with choriocarcinoma after a subsequent full term pregnancy.

According to protocol, all cases received two consolidation courses after normalization of hCG, except for three cases who received only one consolidation course, due to use of an earlier protocol. None of the latter experienced a relapse.

No surgical complications occurred in the intervention group. Side effects of methotrexate occurred in 28 patients (32.6%), comprising 15 patients in the control group and 13 in the intervention group (table 2), with predominantly grade 1 and 2 adverse effects. Two patients in the control group developed grade 3 adverse effects, i.e. severe vaginal bleeding necessitating surgical intervention; one patient underwent emergency uterine curettage and the other underwent hysterectomy on day 6 of the second methotrexate course. In the intervention group one patient requested a hysterectomy when methotrexate failure occurred after the third methotrexate course (see supplementary table 1).

Tables 3 and 4 depict the number of courses needed to reach hCG normalisation in relation to patient characteristics. Linear regression analyses showed that only serum hCG level was statistically significantly related to the number of chemotherapy courses needed. In addition, after adjustment for factors including baseline hCG level, vaginal bleeding, weight and cycle duration in multivariable regression analyses, second uterine curettage did not significantly reduce the number of courses needed to reach normalization of hCG (OR -1.04 95% CI -2.54; 0.46, p = 0.17). There

was also no relation between baseline serum hCG level and uterine dimensions (Pearson correlation coefficient 0.164 (p = 0.132)).

Histopathology after second uterine curettage was available for 42 of 43 cases, revealing molar tissue in 35 patients (83%) and only endometrial tissue in seven patients (17%). The histopathological finding of molar tissue was not predicted by the dimensions of the uterine mass; the mean dimensions for women with molar tissue was 208 ± 293 mm^2, compared to 96 ± 222 mm^2 for women with only endometrial tissue (p = 0.34).

Table 3. Number of courses in relation to patient characteristics

	Methotrexate (n = 43)	**Second curettage and methotrexate (n = 43)**
Bleeding		
• Vaginal bleeding (28 / 23)	4.0 (2.2)	3.5 (1.6)
• No bleeding (15 / 20)	5.1 (2.2)	4.2 (2.9)
hCG pattern		
• Rising (27 / 30)	4.4 (1.9)	3.7 (2.1)
• Plateau (13 / 12)	4.4 (3.0)	3.9 (2.7)
• Persistent (3 / 1)	3.3 (1.5)	4 (--)
hCG level		
• continuous (3000 / 3274)	$5.31 \cdot 10^{-4}$	$4.25 \cdot 10^{-4}$
• < 1500 (15 / 12)	2.8 (1.9)	2.8 (0.8)
• 1500-5000 (28 / 31)	5.2 (1.9)	4.2 (2.5)
Weight		
• continuous (75,6 / 71.5)	0.001 (-0.056, 0.058)	0.03 (-0.04, 0.94)
• ≤ 65 (9 / 15)	4.0 (2.3)	3.4 (1.6)
• > 65 (34 / 28)	4.4 (2.2)	4.0 (2.5)
Mean length of single course		
• continuous (17.9 / 17.9)	-0.03 (-0.20, 0.14)	-0.05 (-0.20, 0.11)
• ≤ 16 days (16 / 19)	3.5 (2.0)	4.0 (2.4)
• > 16 days (27 / 24)	4.9 (2.3)	3.7 (2.2)
Treatment outcome		
• Successful (36 / 37) (*)	4.0 (2.0)	3.3 (1.4)
• second-line treatment (7 / 6)	6.3 (2.6)	6.8 (4.1)
Histology		
1 = Molar tissue (NA / 34)	Not applicable	3.6 (2.1)
2 = No molar tissue (NA / 7)		3.6 (0.5)

(*) "Successful treatment" is defined as reaching normalization of serum hCG level using methotrexate alone, with uneventful follow-up.

"Second-line treatment" is defined as methotrexate failure and subsequent second-line chemotherapy, the need for emergency surgery and treatment for relapse.

Table 4. Linear regression: effect of several factors on number of courses

	OR	95% CI	P value
Curettage	-0.54 (*)	-1.50; 0.43	0.27
hCG level			
• As continuous variable	4.69 ($\cdot 10^{-4}$)	2.32($\cdot 10^{-4}$); 7.07($\cdot 10^{-4}$)	<0.001
• High versus low (^)	1.85	0.88; 2.81	<0.001
Plateau and persistent hCG versus rising hCG	-0.02	-1.05; 1.01	0.97
No bleeding versus vaginal bleeding	0.78	-0.20; 1.75	0.12
weight			
• As continuous variable	0.02	-0.03; 0.06	0.47
• >65 kg versus ≤ 65kg	0.63	-0.44; 1.71	0.24
Cycle duration			
• As continuous variable	-0.04	-0.15; 0.07	0.47
• >16 days versus ≤ 16 days	0.57	-0.41; 1.55	0.25

(*) A negative OR means that fewer courses were needed to reach normalisation of hCG.

(^): low hCG < 1,500 IU / L, high hCG between 1,500 and 5,000 IU/L

Table 5. Linear regression: effect of several factors on treatment success (*)

	OR	95% CI	P value
Curettage	1.20	0.37; 3.91	0.76
hCG level			
• As continuous variable	1.0003($\cdot 10^{-4}$)	1.00($\cdot 10^{-4}$); 1.001($\cdot 10^{-4}$)	0.07
• High versus low	6.64	0.82; 54.0	0.08
Plateau and persistent hCG versus rising hCG	1.28	0.38; 4.32	0.70
No bleeding versus bleeding	1.30	0.40; 4.26	0.66
weight			
• As continuous variable	1.03	0.97; 1.08	0.34
• >65 kg versus ≤ 65kg	1.35	0.4; 5.38	0.67
Cycle duration			
• As continuous variable	1.03	0.91; 1.17	0.63
• >16 days versus ≤ 16 days	2.60	0.66; 10.24	0.17

(*)"Successful treatment" is defined as reaching normalization of serum hCG level using methotrexate alone, with uneventful follow-up. (i.e., 36 out of 43 in the control group, and 37 out of 43 in the intervention group).

Patients were advised to undergo monthly hCG monitoring for a year and to refrain from pregnancy during this period. Three cases were lost to follow-up, and 13 (15.1%) got pregnant within 12 months (table 2). One of them accepted an induced abortion, two suffered first trimester pregnancy loss, one developed a second molar pregnancy, and in one pregnancy the foetus had congenital intestinal obstruction. The eight other pregnancies resulted in a healthy baby.

Treatment success rate was similar for both groups (table 5). After adjustment for hCG level, vaginal bleeding, weight and mean cycle duration, success rates of methotrexate treatment were similar in both treatment groups (OR 0.86 (95% CI 0.24; 3.06, p = 0.81) (table 5).

Discussion

From this randomized study we conclude that second uterine curettage does not significantly impact the number of chemotherapy courses in patients with postmolar GTN. Our results are in line with one retrospective study that concluded that dilatation and curettage of GTN does not affect future chemotherapy requirements [14], and contrast with two other retrospective analyses [6,8]. In a recently published, prospective Gynaecologic Oncology Group study, 26 of 64 (40%) patients did not need chemotherapy after a second curettage [10]. In our study all women were treated with methotrexate, and a possible definitive curative effect of second curettage was not awaited. It is therefore conceivable that the inclusion of women cured by second curettage (i.e., not receiving any chemotherapy) in some retrospective studies led to a lower overall number of chemotherapy courses. However, it should be noted that it is still unclear whether awaiting the curative effect of second curettage affects the total duration of treatment in cases where second curettage fails.

The above mentioned studies suggest that two factors influence the likelihood of reducing the number of chemotherapy courses required to reach hCG normalization, namely the presence of vaginal bleeding and the pre-curettage hCG level. Severe vaginal bleeding can lead to prompt surgical treatment, illustrated in our study by two women in the control arm undergoing emergency surgery for severe bleeding. Since we performed an intention to treat analysis and stratified for vaginal bleeding at presentation, this did not affect the study results. In UK guidelines, the presence of a uterine mass in combination with hCG levels below 5,000 U/L will prompt second curettage [15]. Although all women in the current study had a uterine mass, ultrasound findings did not correlate to the hCG level, nor to the presence of molar tissue at curettage. It is therefore debatable how well the presence of molar tissue is predicted by ultrasonography. While the numbers included in the present study prohibit conclusions on the success or failure of second curettage in the absence of molar tissue in the pathology specimen, it would be reasonable to conclude that second curettage is not beneficial without histological evidence for molar tissue. Although all cases in our study had relatively low hCG levels pre-curettage, initial hCG levels were still related to the number of courses required for normalization. However, when we compared women with hCG levels < 1,500 to women with hCG levels between 1,500 and 5,000, the second curettage did not have an impact on the number of courses needed for normalization of serum hCG levels.

Failure of methotrexate occurred in seven patients and relapse after therapy in four, including a case with a choriocarcinoma after a subsequent full term pregnancy (table 2 and supplementary table 1). Including the two women who underwent emergency surgery, 15% of the patients needed some form of second-line treatment. This is in line with previous reports [16,17].

Limitations of the study:

This study was performed in a single centre, whereas a multicentre setting would be preferable. However, this study and the Gynecologic Oncology Group study are the first prospective studies done, and both showed slow accrual due to the low incidence in most countries. In our study hCG monitoring was carried out at several laboratories, while a standardized test would be preferred. Given local circumstances and resources, the current study therefore reflects daily practice. Although three patients (3%) were lost to follow-up, this seems acceptable given the projected loss of 10%. Fifteen percent of women got pregnant within 12 months, pregnancy outcomes are available for all participants, and therefore it is unlikely that this influenced the outcome of the study.

Methotrexate chemotherapy is generally given in a 2-weekly cycle. In the current study the mean treatment cycle duration was 17.9 days in both groups, and thus unlikely to have impacted the primary study outcome. This observation prompted a substandard care analysis, in which we observed several factors of notice. These included patient-related factors, such as a reluctance to perform blood tests on-time, reluctance to switch to a second-line treatment, difficulties in reaching the clinic, and financial burdens, whereas others were external factors such as poor availability of methotrexate or other second-line drugs in Egypt due to political factors.

Methotrexate is available in vials of 50 mg, and the methotrexate dose in our study was administered in a fixed dose (50 or 75mg), allowing subsequent courses to be administered by a nurse in a home setting. The (international) discussion on the best methotrexate dosing regimen is not yet settled, and some favour a dose related to body weight (1mg/kg) rather than a fixed dose. Since the two treatment groups were comparable regarding body weight and BMI, the regimen and dosing in this study is unlikely to have affected the primary outcome.

Accurate classification of molar specimens into complete or partial moles is important for accurate assessment of the risk of postmolar GTN, with complete moles carrying a significantly higher risk of postmolar GTN (15–20%) than partial moles (0.2–4%) [18-21]. Some reports suggest that partial moles are more common and have higher risk of subsequent GTN in non-western countries [22,23]. Therefore, one of the original secondary endpoints described in the study protocol was the relation

between histopathology and the number of courses needed for normalisation of hCG. However, we decided to refrain from analysing this point, since it was not possible to obtain all original curettage tissues for central pathology review, and secondly, because molecular genotyping was not available [24].

Conclusion

Second uterine curettage has no impact on the number of chemotherapy courses required or on the rate of relapse in low-risk postmolar GTN patients.

Authors' contribution.

HvD was the chief investigator of the trial, RH CB and HvD were the involved in conception and study design. MA, ET, RH were involved in diagnostics, treatment and collection of data. RH, BE-D, EV and HvD were responsible for data analysis and interpretation. RH, EV and HvD were responsible for the preparation and writing of the manuscript. All authors contributed to the manuscript and approved the final manuscript.

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Supplementary table 1: Summary of the failed cases.

Study nr	Age, parity	hCG pattern and level prior to diagnose GTN	Case description	Mean cycle duration (days)	Substand ard care factors
Standard arm: Methotrexate (MTX) treatment (n = 7)					
6	18 y, P1	Rising 4.000	Normalization of hCG after 4 MTX courses, 2 consolidation courses. Relapse 118 days after last MTX consolidation course. Second-line: 3 MAC + 2 consolidation courses.	19.3	3 times treatment delay
30	29 y, P2	Rising 5.000	Normalization after 7 MTX courses, 2 consolidation courses. Relapse 33 days after last MTX consolidation course. Second-line: 2 MAC + 2 consolidation courses.	14.3	None
31	21 y, P3	Rising 4960	Severe vaginal bleeding 6 days after 2nd MTX course, not responding to medical treatment (blood transfusion, haemostatic's) Emergency hysterectomy: histology revealed invasive	16.5	Only one consolidation course

			mole. After hysterectomy she received 2 MTX + 1 consolidation course		
42	30 y, P2	Rising 5000	Failed MTX, plateau after 5 courses. Second-line: 3 EMA/CO + 2 consolidation courses.	18.0	No hCG prior to 2nd and 3rd EMA/CO course.
45	20 y, P0	Plateau 5000	Failed MTX, plateau after 6 courses. Second-line: 3 EMA/CO + 2 consolidation courses.	18.6	Delays in hCG check and MTX start No hCG prior to 2nd and 3rd EMA/CO course.
69	31 y, P1	Rising 2041	Severe vaginal bleeding after 1st MTX course, not responding to medical treatment (blood transfusion, haemostatic's) Emergency curettage: histology revealed invasive mole Significant hCG drop after emergency curettage. After curettage she received 2 MTX + 2 consolidation courses	31.7	Delay due to severe bleeding and bad clinical condition.
77	20 y, P0	Plateau 2059	Failed MTX, rising hCG after 5th course Second-line: 4 EMA/CO + 2 consolidation courses.	17.2	Delays in hCG checks and MTX start No hCG prior to 2nd and 3rd EMA/CO course.
Study arm: second curettage and MTX treatment (n = 6)					
9	27 y P3	Plateau 4.999	Re-curettage: endometrial tissue Normalization after 4 MTX courses, 2 consolidation courses. Received contraceptive pills for 1 year then got pregnant. Delivered normal baby by caesarean section Severe haemorrhage 3 weeks postpartum Curettage: histology revealed choriocarcinoma Second-line: 3 MAC + 2 consolidation courses. Uneventful follow-up for 12 months	16.8	None
15	42 y, P5	Plateau 4.950	Re-curettage: no histology done Failed MTX, plateau after 8th course Second-line: 4 MAC + 2 consolidation courses.	18.1	Plateau should have been recognized after 3rd MTX course.
20	23 y, P1	Rising 5.000	Re-curettage: molar tissue Normalization of hCG after 3 MTX courses, 2	16.0	Treatment protocol violation, according to the protocol EMA/CO or MAC

			consolidation courses. Relapse 78 days after last MTX consolidation course. Second-line: 2 MTX + 2 consolidation courses.		should have been given for second-line treatment.
53	34 y, P3	Rising 5000	Re-curettage: molar tissue Failed MTX, rising hCG after 3rd course Second-line: 3 EMA/CO + 2 consolidation courses.	18.2	No hCG prior to 2nd and 3rd EMA/CO course
60	43 y, P4	Rising 3250	Re-curettage: molar tissue Failed MTX, plateau of hCG after 3 courses, combination chemotherapy was refused, rising hCG after 7th course. Finally combination chemotherapy was accepted. Second-line: 5 EMA/CO + 2 consolidation courses.	15.5	Delay in switch to second-line chemotherapy due to discussions with the patient and family.
64	38 y, P5	Plateau 1328	Re-curettage: molar tissue Failed MTX, rising hCG after 3rd course Tumour board advised EMA/CO or hysterectomy. After counselling hysterectomy was done: histology revealed invasive mole Second-line: 1 EMA/CO + 2 consolidation courses.	19.3	none

Chapter 5

Collaboration benefits all: A commentary

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Most women facing gynaecological cancer live in countries with inadequate health systems and treatment is rarely in line with international standards. This is particularly true for cervical cancer, as most developing countries offer few opportunities for radiotherapy, leaving many women without proper treatment and the risk of avoidable mortality [1]. The Lancet recently highlighted the need to close the global cancer divide for women, but real progress will require not only evidence-based policy making, but also broad multisectoral collaboration and innovative public health approaches to cancer care and control [2]. The gynaecologic malignancy with the highest cure rate is gestational trophoblastic neoplasia (GTN). Postmolar GTN was studied at the Mansoura University Hospital, Egypt, with support from the Erasmus MC Cancer Institute, University Medical Center, Rotterdam, the Netherlands. The outcome of this study has implications for all women with GTN and we would therefore like to share our experiences, and at the same time advocate an increase in worldwide partnerships of researchers to improve our understanding of routine clinical problems.

Outcome of the study in international perspective.

In a randomized controlled trial, we studied the effect of second curettage in postmolar GTN on the number of methotrexate chemotherapy courses, and found that a second curettage did not reduce the number of courses needed to normalize serum human chorionic gonadotropin (hCG) levels [3]. Simultaneously, a multicentre prospective phase II study on the curative effect of re-curettage in a similar patient group, performed in Northern America by the Gynecologic Oncology Group (GOG), showed that a second curettage cured 40% of women with postmolar GTN [4]. Although the studies had different end points, we can conclude that second curettage is of value only when cure (normalisation of hCG) is awaited for. Together, the results of these studies finally settle the question of the role of second uterine curettage in postmolar GTN.

Available collaborations.

After the formation of the Gynecologic Cancer InterGroup (GCIG) the number of high-quality phase 3 trials at a global level have increased. However, most of the involved centres originate in developed countries. Also an analyses on the global patterns of collaboration on meta-analysis showed that most authors are from the larger centres from Northern and Western America and Europe [5]. Developing nations face many health challenges and lack the science, equipment, and financial infrastructure needed for research. Cooperation between the developing and the developed

world is therefore mandatory [6]. The benefits of collaborative projects extend well beyond scientific value alone. In this essay we would like to emphasize several of these "soft" aspects.

Some studies are challenging in the developed world.

Certain studies cannot be efficiently carried out in developed countries due to low disease incidence and decentralized treatment; a case in point is GTN. The difficulty in patient accrual is reflected in the two abovementioned studies; in the GOG study nine centres recruited 64 patients over a period of 5 1/2 years, whereas the single centre Egyptian study recruited 89 patients in 4 years, indicating that international collaboration can sometimes overcome problems of slow patient recruitment [6].

Collaboration improves the standard of care in the low income countries.

Healthcare benefits that may arise from contributing to clinical research include improvements in local infrastructure, the processes of care and the workforce [7]. Due to our involvement in this study, practice at Mansoura Hospital has been evaluated and protocols updated in line with international standards. Other spin offs of this collaboration include the launch of the first trophoblast clinic in Egypt, which now serves as a referral centre for patients in the (wider) area, and the development in Arabic of a patient information leaflet on molar pregnancy. The trophoblast clinic has also established collaborations with sponsors to ensure that care remains available and is provided free of charge. Furthermore, this centre recognized that *recurrent* mole (i.e., three or more episodes of molar pregnancy) occurs more frequently than expected in the Egyptian Nile Delta (incidence figures not yet available). Moreover, collaboration with international experts was sought and chromosomal analyses performed, free of charge, in selected cases. This analysis resulted in the detection of mutations in several patients with recurrent molar pregnancies, and led to the discovery of a recently published novel mutation [8,9].

Collaboration improves the standard of research in the low income countries.

Through these alliances colleagues who are unfamiliar with research practice are encouraged to adhere to international standards, beginning with a well-thought-out research protocol and proper recording of data, preferably using an electronic case record form. By engaging the resources of the supporting parties, such as internet-based randomization programs and access to international literature, the research capacity of colleagues in a low resource setting can be significantly enhanced. Collaborative analysis of data also allows for a better recognition of possible biases and

weaknesses of a study. The knowledge obtained through hands-on support can be transferred to other researchers and staff members, further enhancing the positive effects of a collaborative study. In the case of this particular study, the sharing of results via a consensus workshop on gestational trophoblastic tumours has allowed us to reach gynaecologists, pathologists and oncologists all over Egypt. Finally, the presentation of results at international meetings and publication in high impact journals benefits all researchers concerned.

Collaboration improves mutual understanding.

Persistent GTN is one of the few neoplasias with a very high cure rate, when treated appropriately. However, colleagues in low resource countries often encounter problems unimaginable for professionals in the developed world. A range of challenges can frustrate effective treatment: Economic and political issues may obstruct patient travel to a clinic for appointments, with a resulting unwelcome delay in treatment. Some of these problems are gender related [10], while others require adaptations of (research) protocols to the local situation [11]. Women are more likely to be illiterate than men, and information given to the family may not always be shared with the patient. In many developing countries women cannot or are not permitted by cultural norms to travel by themselves, and therefore rely on a spouse or male family member to reach hospital. Alternatively, women can be treated at home, an approach that might also be of value for women in the developed world who at present have to visit a clinic for methotrexate injections four times within eight days, repeated each cycle. Considerable time and expense might be spared if injections could be given closer to home.

Given the many different national laws and regulations, performing international studies is challenging and costly. However, by performing similar, concurrent studies in multiple countries and uniting the outcome of the various study sites this disadvantage can be partly counterbalanced [6,11,12]. Organizations such as the International Society for the Study of Trophoblastic Disease (ISSTD) could take the lead in organizing these collaborations.

Collaboration is fun.

Last but not least, while international collaborations in which experienced researchers support colleagues in less fortunate circumstances certainly present many challenges, these partnerships can also be both valuable and enjoyable. Collaboration improves local care, offers professional satisfaction and ultimately provides answers to shared scientific and clinical problems.

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Chapter 6

The reproductive outcome after treatment of Gestational Trophoblastic Neoplasia

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Abstract

Background: Treatment of gynecologic cancers in young women is a real challenge as obtaining the future women's fertility is almost always keened by the patient and her family. However, there are reported adverse outcome of pregnancy after treatment.

Objective: To evaluate the obstetric outcome of patients who had undergone fertility preserving treatment for gynecologic premalignant and malignant diseases.

Patients and methods: The study reviewed the pregnancy course and delivery data of 60 patients, who conceived after fertility sparing treatment for gynecologic premalignant and malignant diseases at department of Obstetrics and Gynecology and department of Clinical Oncology & Nuclear Medicine at Mansoura University Hospital from January 2012 to December 2015. We reported pregnancy complications, abnormalities during labor, neonatal outcome and any recurrence after delivery.

Results: The mean age (± SD) of the studied patients was 24.9 (± 6.03) years. The median follow up was 13 months (range: 1-40).The preceding lesions included gestational trophoblastic neoplasia (GTN), 43 cases (71.7%) while early ovarian carcinoma (14 cases, 23.3 %) and CIN III (2 cases, 3.3%) and micro-invasive cervical carcinoma (one case, 1.7%). All cases of GTN were treated with single or multiple agents' chemotherapy. The 3 cases of cervical micro-invasive carcinoma and CIN III were treated with loop electrosurgical excision procedures (LEEP). Cases with stage 1a ovarian cancer were treated with unilateral salpingo-oophorectomy with peritoneal cytology and biopsy of the other ovary. Missed abortion was diagnosed in 4 cases (6.7%). The rate of caesarean delivery (CD) was high (70.9%). Intra-abdominal adhesions during CD were seen in (20.5%) of cases. The neonatal outcome was normal in 53 cases, and 2 cases (3.6%) of had congenital fetal malformations. The poor neonatal outcome was significantly correlated to number of chemotherapy cycles (P=0.04).

Conclusion: The reproductive outcome after fertility preserving treatment of GTN, micro-invasive carcinoma of cervix, CINIII, and stage 1 ovarian cancer is comparable to those of general population. However, there were increased rate of CD, Intra-abdominal adhesions, missed abortion and congenital fetal malformations.

Key words: Gynecologic cancers; reproductive outcome.

Introduction

Treatment of gynecologic cancers in young women represents actually a clinical challenge. Gynecological cancers occur variably at different ages, it may occur at younger age in 12 % of ovarian cancer, 5 % of endometrial cancer, and 2 % of cervix cancer [1].The preservation of the future fertility is usually requested by the patient and desired by her family. Gestational trophoblastic neoplasms (GTN) are well known tumors of reproductive age and mostly chemo-sensitive. However, the rate of recurrent mole, a benign one of this group was reported in 2.5-9% of cases [2]. Gato and colleagues [3] studied the outcome of 50 patients who underwent fertility-preserving treatment for choriocarcinoma, 23 conceived for a total of 43 pregnancies, meanwhile, congenital cardiac defects were observed in 2 of their neonates, namely, ventricular septal defect (VSD) and tetralogy of Fallot.

On the other hand; some cases of cervical cancer occur before the age of 40 years where conservative surgery is to be applied. For these young patients with early stage of the disease, the loop electrosurgical excision procedures (LEEP) and conization provide a hope to preserve their fertilities [4]. Despite radical trachelectomy is considered as a more invasive surgical maneuver; some authors [5] reported in a study involving 210 cases, 35 had live births after this surgery. However, the rate of second trimester miscarriage and preterm labor was recorded to be higher than normal.

Looking to ovarian cancer; conservative surgery remains the ideal for young patients with FIGO Stage I epithelial tumors and also for the borderline tumors [6, 7]. A systematic review carried by Darai et al [8] and concluded that conservative management of BOT resulted in a spontaneous pregnancy rate of 54% for early stage and 34% for advanced stage without increase of the risk of lethal recurrence. Some other authors [9, 10] reported that it is possible to maintain good reproductive function after conservative surgery for ovarian dysgerminoma followed by chemotherapy. Moreover, Vicus et al [11] studied the treatment outcome and reproductive function in women with ovarian immature teratoma and demonstrated the 6 of 11 patients conceived, 3 of them had received chemotherapy.

To the best of our knowledge, there are few published studies in our locality about pregnancy and delivery criteria after fertility preserving treatment of premalignant and malignant gynecologic lesions. So, we conducted this retrospective study.

Patients and methods

We reviewed in a retrospective descriptive manner the subsequent pregnancy and delivery data of 60 patients who conceived after fertility sparing treatment of premalignant and early malignant

gynecologic diseases at the department of Obstetrics and Gynecology as well as department of Clinical Oncology, Mansoura University from January 2012 to December 2015. The study was approved by the Institutional Research Board (IRB) at Faculty of Medicine, Mansoura University.

Patients included were those who had undergone fertility preserving treatment for premalignant and malignant gynecologic diseases, available for follow up during pregnancy and delivery after treatment and those who accept to participate in the study. We excluded patients with incomplete follow up data or inability to contact her or the treating physician.

The data were retrospectively collected by checking the patients' files at MUH and new data recorded by the managing physician outside. The initial criteria of the patient during admission as age, parity, medical condition, diagnosis and stage of the disease, surgical intervention if any, postoperative chemotherapy (type and number of courses) and follow-up data were preliminary registered. The data about subsequent pregnancy and delivery were collected by contacting the patient and her managing colleague at our center or at the private clinics where we reported pregnancy complications, type and abnormalities during labor, neonatal outcome and also any recurrence after delivery. All gathered data are then subjected to statistical analysis.

Statistical analysis.

All data collected were statistically analyzed by using SPSS for windows version 17.0 (SPSS, Chicago, IL). Continuous data were expressed as mean ± standard deviation (SD) and proportions for the socio-demographic characteristics. Multivariate analyses were performed where outcome of pregnancy was the dependent variable and number of cycles as well as interval between chemotherapy and pregnancy were independent variables. Mann-Whitney test and Kruskal-Wallis test were adopted to analyze the differences under different items. . P-value equal or <0.05 was set statistically significant.

Results

Sixty patients got pregnant after fertility preserving treatment of premalignant and malignant gynecologic lesions. The median follow up period was 13 months (range: 1-40).The mean age ± SD of our patients was set 24,9 ± 6,03 years meanwhile the median parity was 1.0 (range:1-4). The mean time interval from surgery or last cycle of chemotherapy till pregnancy ± SD was 14.47 (8.21) months and ranged from (1- 40) months. The predominant recorded lesions were gestational trophoblastic neoplasia (43 cases, 71.7%) followed by early ovarian carcinoma (14 cases, 23.3 %), lastly CIN III (2 cases, 3.3%) and micro-invasive cervical carcinoma (one case, 1.7%). The initial diagnosis and FIGO stage of the disease were presented and obviously all patients were in stage 1 except for 2 cases with cervical intraepithelial neoplasia (CIN III), table (1). In addition; the same

table represented the fertility preserving treatment of the studied cases. Low-risk GTN cases were treated with methotrexate (50 mg/kg body weight) alternating with folinic acid (0.1mg/kg) but 5 cases failed to respond, so they received multiple agent chemotherapy (EMACO) together with one case of high-risk GTN. Three cases of cervical microinvasive carcinoma and CIN III were treated with LEEP. Furthermore, cases with stage 1a ovarian cancer were treated with unilateral salpingo-oophorectomy with peritoneal cytology and biopsy of other ovary. Three cycles of adjuvant chemotherapy (Carboplatin-Paclitaxel) were added to patients with stage 1c epithelial tumors and 4 cycles of (Bleomycin-Etoposide-Cisplatinum) for stage 1c germ cell tumors.

Table (1) : Patients' demographic data.

Variable	**Total number (60)**			
Age in years (mean & SD)	24.92 (6.03)			
Parity (median & range)	1.0 (1 - 4)			
Mean interval from treatment to pregnancy in months (SD)	14.47 (8.21) **Range:** (1-40) months			
Type of tumor:	**Number (%)**	**FIGO stage**	**Treatment offered**	
			Surgery	**Chemotherapy**
1. GTN	43 (71.7)	1 (43 cases)	No	Methotrexate (37),
2. Ovarain:				EMA/CO (6)
Borderline tumors	1 (1.7)	1a (1 case)		
Serous cystadenocarcinoma	5 (8.3)	1a (3 cases)	USO	No
		1c (2 cases)	USO	No
Mucinous	2 (3.3)	1a (1 case)	USO	Carboplatin-Paclitaxel
cystadenocarcinoma		1c (1 case)	USO	No
	2 (3.3)	1a (1 case)	USO	Carboplatin-Paclitaxel
Immature teratoma		1c (1 case)	USO	No
			USO	BEP
	1 (1.7)	1c		
Mixed GCT				
	1 (1.7)	1a (1 case)	USO	BEP
Dysgerminoma	2 (3.3)	1a (2 cases)		
Granulose CT			USO	No
	1 (1.7)		USO	No
	2 (3.3)			
3. Cervical carcinoma		1a1 (1 case)	LEEP	No
4. CIN III		0 (2 cases)	LEEP	No
Mean cycles of chemotherapy(SD)	4.15 (2.08)			

Abbreviations: USO; unilateral salpingoopherectomy, LEEP; loop electrosurgical procedure, EMACO; Etoposide, Methotrexate, Adriamycine, Cyclophosphamide, and Vincristine, BEP; Bleomycin, Etoposide and Cisplatinum**.**

Table [2]: Pregnancy and labor courses among the studied cases

Variable	Number (%)
Pregnancy course: (n=60)	
FTNP	51 (85%)
PIH	1 (1.7%)
Preterm pregnancy	3 (5%)
Missed abortion	4 (6.6%)
Induced abortion	1 (1.7%)
Labor course: (n=55)	
VD	16 (29.1)
CD	39 (70.9)
Findings during CD: (n=39)	
-Intraoperative adhesions	8 (20.5%)
-Congested uterus	2 (5.1%)
-Thin uterine wall	1 (2.6%)
-Caesarean hysterectomy	2 (5.1%)
Neonatal outcome: (n=55)	
Normal	53 (96.4%)
CFMF	2 (3.6%)
Recurrence after delivery:	1 (1.7%)

Abbreviations: FTNP; full term normal pregnancy, PIH; pregnancy induced hypertension, VD; vaginal delivery, CD; cesarean delivery, CFMF; congenital fetal malformation.

Table [3]. Correlation of missed abortion and neonatal outcome to chemotherapy.

	Pregnancy		P value	**Neonate**		P value
	Normal	Missed abortion		Normal	CFMF	
1.Number of cycles Median (range)	4(1-10)	5(2-6)	0.17	4(1-9)	8(6-10)	0.04
2.Interval* Median (range)	13(1-40)	9(3-13)	0.049	13(1-36)	13(8-18)	0.23

Interval*: From surgery or last cycle of chemotherapy till pregnancy.

Small number of cases in adverse pregnancy and neonatal outcome might result in statistical inaccuracy.

The pregnancy outcome was presented in table (2). There were 3 cases (5%) of preterm labor at 36 weeks, none of them needed incubator care, 2 of them had performed LEEP whilst one after treatment of GTN. There were 4 cases of missed abortion (6.7%), 3 of them were after chemotherapy, and a case of induced abortion (due to short interval between chemotherapy and pregnancy). The occurrence of missed abortion was significantly correlated to shorter interval between chemotherapy and pregnancy (P = 0.049). No reported maternal complications during

pregnancy except for pregnancy-induced hypertension in one case. Regarding delivery; 55 cases were delivered, 39 (70.9%) delivered by caesarean delivery (CD) and 16 (29.1%) delivered vaginally. Two cases of ovarian tumors (stage 1c) performed total abdominal hysterectomy and salpingo-oophorectomy with surgical staging during CD under request of the patients. However, there was no evidence of disease recurrence after histopathological examination of their specimens. During caesarean delivery, 8 of 39 cases (20.5%) had adhesions between the uterus and intestine. Seven of them had undergone conservative surgery for ovarian cancer and one case received combination chemotherapy for GTN. Two of 39 cases (5.1%) had congested uterine wall with edema and tearing of the stitches. One case received single whereas the other received combination chemotherapy. Furthermore, one case (2.6%) previously treated choriocarcinoma with combination chemotherapy; the fundus of the uterus was found to be thin which probably the site of the initial tumor. Biopsies from the other ovary, adhesions, omentum, and peritoneum were taken during CD and proved free (Table 2). The neonatal outcome was normal in 53 cases, and 2 cases (3.6%) had congenital fetal malformation (both received chemotherapy).One case of them had congenital intestinal obstruction (received 10 courses of methotrexate) and the other case of ventricular septal defect (received 6 courses of EMACO). The poor neonatal outcome was significantly correlated to number of chemotherapy cycles (P=0.04).However, neonatal outcome was not correlated to interval between pregnancy and chemotherapy (P=0.2) (table 3). No reported cases of recurrence after treatment of cervical and ovarian lesions in our study. Only one case of GTN recurrence was reported after one month of delivery (1.7%). She was presented by secondary postpartum hemorrhage and received EMACO protocol (table 2).

Discussion

During the decision making for gynecologic cancer, the tumor board usually faces a challenge between the both oncological and reproductive outcomes. This particularly occurs when the gynecologic cancer patient is at young age and needs a future pregnancy. This raised our attention for retrospective analysis of 60 patients who conceived after fertility preserving treatment for GTN, microinvasive cervical carcinoma, CIN III, and early ovarian cancer.

It is well known that, the prognosis of GTN after fertility sparing treatment is excellent as the tumor is chemosensitive. Single agent Methotrexate is used as first line for low risk cases and EMACO is the commonly used second-line combination chemotherapy protocol [12,13]. We reported that pregnancy following chemotherapy resulted in 4 missed abortions (6.7%) and one case of induced abortion. The adverse pregnancy outcome for patients was not correlated to number of chemotherapy cycles (P=0.17) but was significantly correlated to the shorter interval between the last chemotherapy and pregnancy (P=0.049). The miscarriage after chemotherapy was also reported

by Gadducci et al [14].The long term effect of chemotherapy on obstetric outcome is unclear and needs to be evaluated in a large prospective study. Moreover, one case developed recurrence one month after delivery which necessitated continuing postpartum surveillance of the cured patients.

In our study 3 cases had undergone LEEP procedure for treatment of CIN III and microinvasive carcinoma, however 2 of them developed preterm labor. The association between LEEP and preterm births was also proved by Bjorge et al [15].The oncological and reproductive outcome of the managed 14 cases of early ovarian cancer were relatively excellent as the authors did not report any case of recurrence during the follow up period and with a satisfactory neonatal outcome even after receiving adjuvant chemotherapy, this comes similar to results found by many other authors [6-10].

The mode of delivery of the studied cases was CS in 39 (70.9%) which is considered a very high figure compared to the reported national figures (22%) [16].The high rate of CS may be attributed to the concept of the treating physician to perform "second-look" during CS or request of the patient due to fear of limited fertility after surgery and chemotherapy. However, this explanation was not supported by other authors.

Regarding the neonatal outcome in our study, we reported 2 cases (3.6%) of congenital fetal malformation (both received chemotherapy). This rate is higher than general population which reported to be 2.4% [17]. The poor neonatal outcome was significantly correlated to the number of chemotherapy cycles (P=0.04) (table 3).This finding is supported by some other study done by Gato et el [3] who reported that total dose of methotrexate was higher in patients who delivered a child with cardiac anomaly. However, our finding did not agree with Gadducci et al [18] who reported that there was no increase of risk of CFMF after chemotherapy for GTN. This disagreement may be due to small sample size of our study.

One limitation of this study is lack of data about the total number of cases who had underwent fertility preserving treatment during the study period, so we could not estimate the ratio of pregnancy among the patients who had received the same treatment. Small number of cases with cervical lesions as well as adverse pregnancy and neonatal outcome might result in statistical confusion. Lastly; the authors also recommend a multicenter not a single center study, like ours, for the results to be more clarified and satisfactory.

Conclusion

Fertility-sparing treatment is a viable tool to enable gynecological cancer patients of young age to fulfill their family building without impairment of oncological outcome and the reproductive outcome of GTN, micro-invasive carcinoma of cervix, CINIII and stage 1 ovarian cancer is

comparable to those of general population but with increased rate of CD, Intra-abdominal adhesions, missed abortion and congenital fetal malformations.

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Chapter 7

Prevalence of Gestational Trophoblastic Diseases after Histopathologic Examination of Specimens of Pregnancy Termination and Post-abortive Bleeding

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Abstract

Objective: To determine the prevalence of GTD in the referred specimens of uterine evacuation after miscarriage, in clinically diagnosed molar pregnancies, and post-abortive bleeding.

Methods: The referred clinical reports to Pathology department of Mansoura University& private practice settings and their corresponding histopathologic diagnoses during the period from 1/1/2009 to 31/3/2014 were reviewed.

Results: The study included 640 referred specimens of contents of uterine evacuation. The mean age of the cases was 26.5 years (range: 15.0-54.0 years). The mean GA was 10.3 weeks (range: 5.0-19.0 weeks).The commonest clinical diagnosis of the referred cases was missed abortion (329 cases, 51.4%). Molar pregnancy was diagnosed histologically in 103 of 499 referred cases as miscarriage (20.6%). Histopathological examination of specimens of uterine curettage due to post-abortive bleeding revealed GTN in 12 of 27 cases (44.4%).

Conclusion: Molar pregnancy was diagnosed histologically in 20.6% of the referred cases as various types of miscarriage. We recommend histopathologic examination of uterine contents after pregnancy termination and post-abortive bleeding. Further studies are needed to confirm this high prevalence of molar pregnancy in our locality.

Key Words: Miscarriage; Pathology; Molar pregnancy.

Introduction.

Gestational trophoblastic disease (GTD) includes several disease processes that originate in the placenta. Under the WHO classification, gestational trophoblastic disease includes hydatidiform mole, invasive mole, choriocarcinoma, placental site trophoblastic tumors, and epithelioid trophoblastic tumors [1]. The majority of both complete and partial hydatidiform moles present as early pregnancy failure with or without vaginal bleeding [2]. This mimics miscarriage, the most common complication of pregnancy, occurring in 15-20% of all pregnancies [3]. Not all women with a miscarriage will consult a gynecologist, or undergo ultrasonography. Even when women visit a gynecologist because of symptom of bleeding in early pregnancy the diagnosis of a molar pregnancy can be missed by clinical and ultrasound examination. Fowler et al [4] concluded from their large retrospective study that routine pre-evacuation ultrasound examination identified less than 50% of hydatidiform moles. Detection rates in their study were higher for complete compared to partial moles.

Routine histopathologic assessment of products of first-trimester miscarriages may therefore diagnose important pathologies such as molar pregnancy and placental site trophoblastic disease [5]. But in many cases, histopathological examination of the products of conception is not performed.

Post molar GTD was reported to occur in 7.5-20% of patients after evacuation of complete hydatidiform moles and in 2.5-7.5% following evacuation of partial moles. The vast majority of cases occur during the first 6 months after molar evacuation [6].

There is worldwide geographic variation in incidence of GTD. The incidence of molar pregnancy in southeast Asia was reported to be 1-12 per 1000 pregnancies which is 7-10 times higher than that in Europe or North America where it is reported to be 0.5-1 per 1000 pregnancies [7,8,9].In Africa, the incidence of molar pregnancy and choriocarcinoma was 1.2 and 0.5 per 1000 deliveries, respectively [10].

The aim of this retrospective study is to determine the prevalence of GTD in the referred specimens of uterine evacuation after miscarriage, in clinically diagnosed molar pregnancies, and post-abortive bleeding.

Patients and Methods.

This retrospective study was performed in the department of Pathology and department of Obstetrics and Gynecology, Mansoura University as well as private practice settings. The referred

clinical reports and their corresponding histopathologic diagnoses in the period from 1/1/2009 to 31/3/2014 were reviewed.

The clinical data were collected from the referral letters (age, gestational age, and ultrasound diagnosis). Histopathological diagnoses were obtained by an expert gynecologic pathology team after hematoxylen and eosin staining. In doubtful cases, the diagnoses were confirmed by immunohistochemical staining.

Inclusion criteria.

Referred specimens of products of uterine evacuation with a gestational age of 19 weeks or less (spontaneous, missed, and incomplete miscarriage), referred specimens as products of uterine evacuation of clinicaly (and by ultrasound) diagnosed molar pregnancy, and post-abortive bleeding in which initial specimens of miscarriage were not examined.

Exclusion criteria.

Cases with miscarriage who were managed by expectant treatment with no available specimens and referred cases that were clinically diagnosed as ectopic pregnancy.

The histopathologic diagnosis was compared to the clinical (including ultrasound) diagnosis as stated by the gynaecologist who referred the patient. Hospital files were retrieved for cases with histopathologic diagnosis GTD, to obtain all clinical and follow up data.

Statistical analysis.

Data was analyzed using SPSS (Statistical Package for Social Sciences) version 15. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data was presented as mean ± SD. $P < 0.05$ was considered to be statistically significant.

Results

The study included 640 referred specimens of contents of uterine evacuation after early pregnancy loss, clinically diagnosed molar pregnancies, and post-abortive bleeding. The mean age of the cases was 26.5 years (range: 15.0-54.0 years). Gestational age (GA) was available in 613 cases. The mean GA was 10.3 weeks (range: 5.0-19.0 weeks).

The commonest clinical diagnosis of the referred cases was missed abortion (329 cases, 51.4%). Molar pregnancies, spontaneous abortion, incomplete abortion and post abortive bleeding represented 114 cases (17.8%), 96 cases (15%), 74 cases (11.6%), and 27 cases (4.2%) respectively.

Table 1 summarizes the correlation of the clinical diagnosis to the histopathological diagnoses of the referred cases. There were 499 cases referred to the pathologist as various types of miscarriage (missed, incomplete, and spontaneous miscarriage), the pathologic diagnoses of these cases revealed that 103 of them (20.6%) were complete or partial mole.

Table 1. Histopathological diagnoses of uterine contents in different clinical types of abortions.

	Products of conception	**Decidual reaction&IER***	**Partial mole**	**Complete mole**	**Other GTD**	**Total**
Missed abortion**	221 (67.2%)	39(11.9%)	65 (19.8%)	4(1.2%)	0	329
Incomplete abortion	45(60.8%)	13(17.6%)	5 (6.8%)	10(13.5%)	1 (1.4%), CC#	74
Spontaneous abortion	63(65.6%)	14 (14.6%)	13(13.6%)	6(6.3%)	0	96
Molar pregnancy	12(10.5%)	1(0.9%)	37(32.5%)	64(56.1%)	0	114
Post abortive bleeding	9(33.3%)	6(22.2%)	0	4(14.8%)	8(29.6%), 5 cases of CC# 2 cases of PSTT## 1 case of invasive mole	27
Total	**350**	**73**	**120**	**88**	**9**	**640**

IER*: Irregular endometrial response.

Missed abortion**: including recurrent missed abortion. CC#: choriocacinoma.

PSTT##: placental site trophoblastic tumor

Table (2) Correlation of the age of the patient to development of GTN.

Age group	**Non GTN**	**GTN**	**Total**
<20y	101	16 (13.7%)	**117**
20-30y	371	13 (3.4%)	**384**
30-40y	107	7 (6.1%)	**114**
>40y	18	7 (28%)	**25**
Total	**597**	**43**	**640**

Chi-Square Tests

	Value	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	33.968	0.0001

As can be seen from table 1; the clinical and ultrasound diagnosis "molar pregnancy" was confirmed pathologically in 101 of 114 cases (88.6%). In the other 13 cases (11.4%) the clinical diagnosis molar pregnancy was not confirmed by histopathology.

Post-abortive bleeding represented 27 (4.2%) of the referred cases, 12 of them (44.4%) were found to be GTD, including five cases of choriocarcinoma, two cases of PSTT, one case of invasive mole, and four cases of vesicular mole (table 1). For none of the 27 cases, histopathological examination of products of conception was performed at the time of miscarriage. Uterine curettage was performed 7- 42 days thereafter.

The progression to GTN was significantly correlated to the extremes of the reproductive age of the patient ($P < 0.0001$). The correlation of age of the patient to development of post-molar GTN is represented in table 2.

Discussion

It is often difficult to differentiate between retained products of conception and GTD solely on the basis of clinical criteria. Furthermore, the sonographic appearance of products of conception can share similar imaging findings with early GTD [11]. Histopathological examination of products of conception remains the current gold standard for the detection of gestational trophoblastic diseases [4,12,13].

The study included 640 referred specimens of contents of uterine evacuation after early pregnancy termination and post-abortive bleeding. The histopathological diagnoses were correlated to the pre-evacuation clinical and ultrasound data as supplied to the pathologists through the referred clinical reports.

Partial and complete molar pregnancies were diagnosed histologically in 103 of 499 referred cases as products of uterine evacuation of miscarriage (20.6%). This figure is much higher than reported by Tasci et al [2] who reported that by histopathologic examination of products of conception partial hydatidiform mole was diagnosed in 2.1%, complete hydatidiform mole in 0.43% and placental site trophoblastic tumor was detected in 0.12%. This discrepancy may be due to larger sample size of their study (1606 cases versus 499 cases in our series).Other explanation of overestimation of molar pregnancy in our study that we only included cases who referred for histopathological assessment and not all cases of miscarriage. A third reason is the different incidence of GTD with different geographic distribution.

The histopathological examination confirmed the pre-evacuation clinical and ultrasound diagnosis of molar pregnancy in101 of 114 cases (88.6%). Again, this figure is higher than reported by Fowler et al [4], who reported that routine pre-evacuation ultrasound examination identified less than 50% of hydatidiform moles. The difference in our figures may be due to the selection bias; our center is a tertiary referral center and including the high number of patients with clinical molar pregnancy influenced this percentage.

Molar pregnancy was more common in extremes of reproductive age in our cases ($P < 0.0001$), this finding was also reported by Bracken [14].

Histopathological examination of specimens of uterine curettage in cases of post-abortive bleeding diagnosed 12 of them (44.4%) as GTD. The high incidence of GTD among studied cases with post-abortive bleeding arouses the importance of mandatory histological examination of uterine contents of this group of patients. To the best of our knowledge; this finding was not reported by other authors.

There is no doubt that many cases of pregnancy termination in our locality were not subjected to histopathological examination. The authors tried to investigate for the cause. The most important cause was that the clinician was satisfied with his /or her clinical and ultrasound diagnosis. Other causes were presentation of some patients with emergency vaginal bleeding and non-availability of pathology laboratories in the rural areas. Furthermore, expectant management of miscarriage decreased the chance of specimen examination because in most cases the products of conception were expelled at home. Expectant management of miscarriage was recommended by many authors [15,16,17].

Although ideally all specimens removed should be assessed by histopathology, routine assessment of miscarriage will be costly and time consuming. We should at least try to identify those cases that are at risk of gestational trophoblastic disease; as women in the extremes of reproductive age, women with remarkable findings on ultrasound, and women with recurrent miscarriage.

The limitations of this study are scanty clinical data in the referred clinical reports and its inclusion only cases that referred for histopathological assessment and not all cases of miscarriage and post-abortive bleeding which overestimated the prevalence of GTD in the studied cases.

Conclusion

Unsuspected molar pregnancy was diagnosed histologically in 103 of 499 referred cases as miscarriage (20.6%). Histopathological examination of specimens of uterine curettage due to post-abortive bleeding revealed GTD in 44.4% of cases. We recommend histopathologic examination of

uterine contents after pregnancy termination and post-abortive bleeding. Further studies are needed to confirm this high prevalence of molar pregnancy in Egypt.

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Chapter 8

Expression of p57Kip2 in Early Molar Pregnancies and Their Relations to the Progression to Persistent Trophoblastic Disease.

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Abstract

Background: Although the morphological features characteristic of products of conception specimens including molar pregnancies are well described, substantial histopathological similarities are observed between the different entities, especially in cases of early pregnancies. Furthermore, there are no current solid criteria that could predict cases with progression to persistent gestational trophoblastic disease. In this study, we aimed to determine the most specific histopathological and immunohistochemical features required for accurate diagnosis that can reliably predict the clinical behavior.

Methods: Sixty-five cases of products of conception were reviewed clinically and pathologically, and any progression to persistent gestational trophoblastic disease (PGTD), if present, was noted. Pathological assessment of the archival material included re-cut sections of 5 μm in thickness, routine staining with hematoxylin and eosin and immunohistochemical staining of p57Kip2.

Results: Certain histopathological criteria were found to be significant in differentiation between complete hydatidiform mole (CHM) and partial hydatidiform mole including villous shape and outline, villous trophoblast hyperplasia, and atypia in extravillous trophoblasts. There were no significant differences in any morphological or immunohistochemical features between cases with or without subsequent development of GTD.

Conclusions: Histopathological diagnosis of molar pregnancy remains problematic especially in early gestation. Their diagnosis should be stated after a constellation of specific histopathological criteria in order not to miss CHM. p57Kip2 immunohistochemistry is of great value in diagnosis of cases that had equivocal morphology by histopathological examination. However, there were no significant features to predict cases that subsequently developed persistent GTD.

Key Words: Hydatidiform mole; Complete hydatidiform mole, early; p57Kip2 immunohistochemistry

Introduction

Hydatidiform mole (HM) is an abnormal gestation characterized by significant hydropic change and variable trophoblastic proliferation involving part or all of chorionic villi [1,2]. HM is categorized into two separate entities, complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM), according to morphology and cytogenetics. However, hydropic abortion (HA) could morphologically mimic HM [3,4].

Characteristic morphologic features have been proposed for the diagnosis of product of conception (POC) specimens including CHM, PHM, and HA. However, substantial histopathological similarities are observed between the three entities, especially in cases of early detection resulting in interobserver and intraobserver variability in the diagnosis of products of conception (POC) specimens [5,6]. Thus, the diagnosis of POC on the basis of histopathology alone remains a challenge for pathologists, even those experienced [7].

Persistent gestational trophoblastic disease (PGTD) develops after CHM in 10% to 30% of cases,[4] and after PHM in 0.5% to 5% whereas HA has no relation to PGTD [5]. Thus, follow-up serum β-human chorionic gonadotropin (β-HCG) measurements aren't essential in cases of HA, whereas these measurements form part of surveillance for PGTD in cases of HM. Therefore, accurate distinction of CHM from PHM on one hand and of HM from HA on the other hand is important for appropriate clinical management and has prognostic implications [3].

Difficult cases may require molecular techniques using the differences in DNA content of different POC cases [8]. However, such molecular diagnostic methods are technically difficult, relatively costly, and non-available in most of pathology laboratories [2]. Detection of the expression of gene products such as p57Kip2 by the trophoblastic cells should be highlighted. The p57Kip2 gene (CDKN1C) is a strongly paternally imprinted gene expressed only by the maternal allele in most tissues and is involved in implantation [9]. In normal placenta, nuclear p57Kip2 expression is seen in villous cytotrophoblast, extravillous trophoblast, villous stromal cells, and deciduas [10,11]. p57Kip2 can identify CHM (androgenetic diploidy) by the lack of its expression12-14 and can be helpful in distinguishing CHM from PHM and non molar HA,11 but can't distinguish PHM (diandric monogynic triploidy) from nonmolar (biparental diploidy) specimens as both are positive [14]. CHM mostly doesn't contain maternal genome. Therefore, p57Kip2 staining is unexpressed or greatly reduced in the nuclei of their cytotrophoblasts and stromal cells [11]. Products of conception containing maternal genetic material, PHM and nonmolar HA, show positive nuclear p57Kip2 staining in cytotrophoblast and villous stromal cells. p57Kip2 is also expressed in intermediate trophoblast islands and decidual cells, serving as positive internal control in all POC cases [12,13].

In this study, we aimed to define precise histopathological and immunohistochemical features to make a proper diagnosis of the different types of HMs especially in early gestational ages and to distinguish those from other mimics. Furthermore, specific features to predict prognosis and progression to PGTD were investigated.

Materials and Methods

Sample collection

Archival materials of sixty five cases of POC specimens were retrieved from the department of Pathology, Faculty of Medicine, Mansoura University, between January 2013 and December 2014. The protocol was approved by the Ethical Committee of Mansoura University and informed consents were obtained from the patients.

Histopathological review

The paraffin blocks were re-cut in 5-μm-thick sections and stained with hematoxylin and eosin stain and were independently reviewed by two pathologists for evaluation of the main morphological findings of HM.[13,15]

Immunohistochemistry

Five-micrometer-thick tissue section from each case was subject to immunohistochemical staining using monoclonal antibody against the p57Kip2 protein (NeoMarkers/Lab Vision Corpora- tion, Fremont, CA, USA) with dilution 1:200 and the Envision system (DakoCytomation, Glostrup, Denmark). All the steps were performed according to the manufacturers' instructions.

In all cases, p57Kip2 was assessed in the nuclei of the villous cytotrophoblasts, extravillous trophoblasts, and villous stromal cells. Specimens were interpreted as positive for p57Kip2 staining when there was distinct nuclear staining of villous cytotropho-blasts and stromal cells. The p57Kip2 stain was interpreted as negative when there was no distinct staining or limited nuclear staining (< 10%) of villous cytotrophoblasts and stromal cells. Staining of intermediate trophoblasts and/or maternal decidua was considered as the positive internal control for these specimens.

Statistical methods

Data were analyzed using the program SPSS ver. 20 (IBM Corp., Armonk, NY, USA) to obtain descriptive statistics. Statistical significance was determined at 95% level of confidence (i.e., differences will be considered significant if $p<0.05$).

Results

Examination of clinical data

The age of patients ranged from 16 to 50 years with a mean of 26 ± 8 years and a median of 24 years. All cases were in the first or early second trimester. Their gestational age ranged from 6 to 14 weeks with a mean of 9±2 weeks and a median of 8 weeks.

Histopathological examination

Significant histopathological criteria for the diagnosis and distinguishing different entities are shown in Table 1. Examples of the morphological features are demonstrated in fig. 1.

Immunohistochemistry

Specimens that showed distinct nuclear staining of cytotrophoblasts and villous stromal cells were interpreted as positive for p57Kip2 (Fig. 1H). On the other hand, specimens that showed no or limited nuclear staining (in < 10%) of cytotrophoblasts and villous stromal cells with positive internal control were considered negative for p57Kip2 stain (Fig. 1). Accordingly, the studied cases were redistributed as shown in table 2.

CHM originally constituted 39 cases. However, after reevaluation based on the histopathological criteria and p57Kip2 immunohistochemistry, seven cases were added to CHM (previously diagnosed as PHM). On the other hand, one case originally diagnosed as CHM was converted to PHM. The final typing of the molar cases was, therefore, 45 cases of CHM and 11 cases of PHM.

Table 1. Significant histopathological criteria according to the final diagnosis of the studied cases using p57Kip2 immunohistochemistry

Significant criteria differentiating between CHM and PHM	Significant criteria differentiating between molar and nonmolar pregnancy
Villous shape and outline: p < .001	Villous shape and outline: p < .001
Villous trophoblast hyperplasia: p = .001	Cistern formation: p < .001
Atypia at extravillous trophoblast: p < .001	Trophophoblastic inclusion: p = .001
	Villous trophoblast hyperplasia: p < .001

CHM, complete hydatidiform mole; PHM, partial hydatidiform mole.

Table 2. Comparison of the diagnosis of the studied cases by his- topathological examination and p57Kip2 immunohistochemistry (n= 56)

	Case		p-value
Method of diagnosis	**CHM**	**PHM**	
Haematoxylin and eosin stain	39 (60)	17 (26)	<.001
p57Kip2 immunohistochemistry	45 (69)	11 (17)	

Values are presented as number (%).

CHM, complete hydatidiform mole; PHM, partial hydatidiform mole.

Follow-up

By following the clinical history of all studied cases, nine cases (14% of total/16% of molar cases) were found to progress into gestational trophoblastic disease (GTD) based on persistence of symptoms and serum β-HCG level (table 3). These represented 18% of CHM and 9% of PHM cases. As shown in Table 4, his-topathological criteria of these cases were examined and compared with the results of p57Kip2 immunohistochemistry to detect the most statistically significant histopathological feature that can predict the progression of molar disease into PGTD. According to the current study, none of the studied histopathological parameters could differentiate between cases with or without progression to GTD.

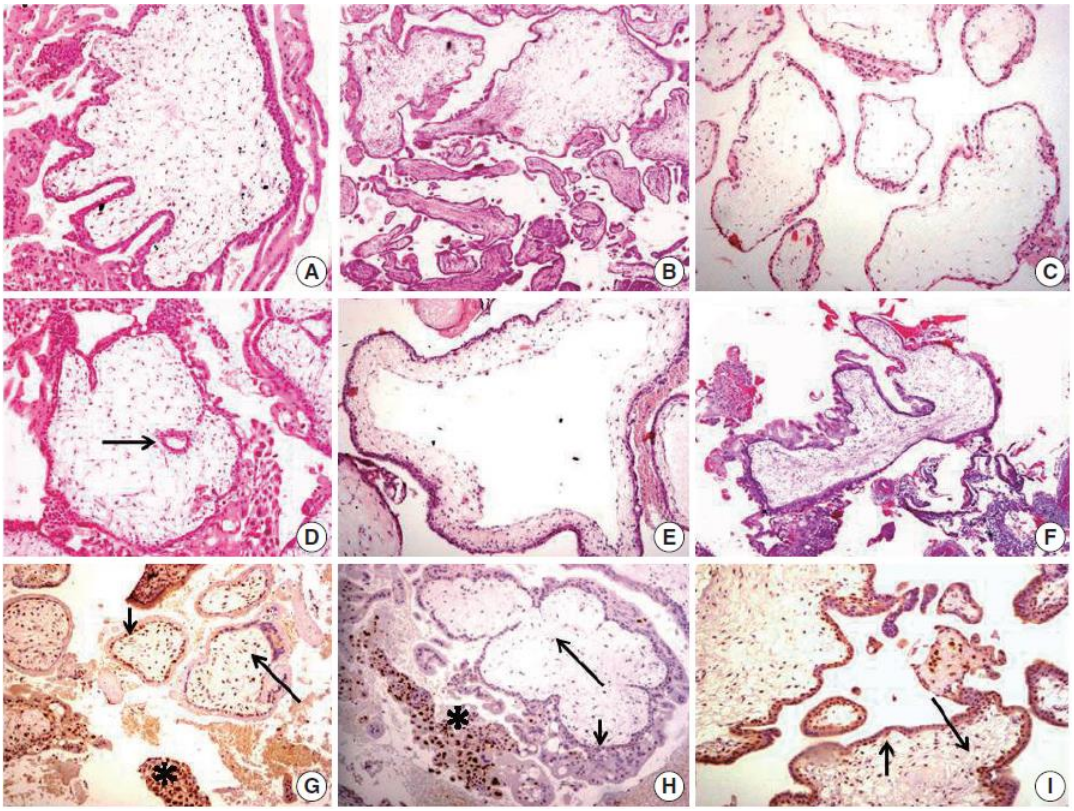

Fig. 1. (A) Complete hydatidiform mole (CHM): irregular villous outline (club shaped) with villous stromal karyorrhectic debris. (B) Partial hydatidiform mole (PHM). Two villous populations; large edematous villi with irregular outline and normal appearing nondistended ones. (C) Hydropic abortion (HA): distended villi with hydropic change. (D) CHM: trophoblastic inclusion (arrow). (E) CHM: cistern formation. (F) CHM: multifocal villous trophoblastic hyperplasia. (G) HA: positive p57Kip2 nuclear staining in intermediate trophoblast as positive internal control (asterisk). Positive p57Kip2 nuclear staining in villous cytotrophoblasts (CT) (short arrow) and villous stromal cells (long arrow) (immunoperoxidase). (H) CHM p57Kip2 immunohistochemistry: negative nuclear staining in villous CT (short arrow) and villous stromal cells (long arrow); positive p57Kip2 nuclear staining in intermediate trophoblast as positive internal control (asterisk) (immunoperoxidase). (I) PHM: positive p57Kip2 nuclear staining in villous CT (short arrow) and villous stromal cells (long arrow) (immunoperoxidase).

Table 3. Cases that developed gestational trophoblastic neoplasia (n = 9)

Case	Age (yr)	GA (wk)	Histopathology	p57Kip2	β-HCG (mIU/mL)	MTX	Response	Second-line treatment
1	20	10	CHM	Negative	2907	2	Yes	-
2	28	4	CHM	Negative	1294	2	Yes	-
3	29	8	CHM	Negative	280	1	Yes	-
4	48	8	CHM	Negative	1328	3	No	Hysterectomy
5	20	12	CHM	Negative	4177	3	Yes	-
6	29	9	CHM	Negative	446	2	Yes	-
7	19	8	CHM	Negative	3260	2	Yes	-
8	42	10	CHM	Negative	5000	6	No	EMA/CO
9	24	8	PHM	Positive	4990	5	Yes	-

GA, gestational age; β-HCG, β-human chorionic gonadotropin; MTX, methotrexate; CHM, complete hydatidiform mole; EMA/CO, etoposide, methotrexate, actinomycin D, cyclophosphamide, vincristine/oncovine; PHM, partial hydatidiform mole.

Table 4. Histopathological criteria of cases that clinically progressed to PGTD in comparison to the rest of the series

Parameters		PGTD (n = 9)	Non-PGTD (n = 56)	p-value
Villous shape and outline	Distended regular	1	9	> .99
	Distended irregular	5	24	
	Non-distended	1	8	
	Two population of villi (distended and non-distended)	2	15	
Cistern	Present	8	45	> .99
	Absent	1	11	
Trophoblastic inclusion	Present	6	34	> .99
	Absent	3	22	
Stromal myxoid change	Present	4	26	> .99
	Absent	5	30	
Villous trophoblast hyperplasia	Circumferential	4	21	> .99
	Multifocal	4	27	
	Polar	1	8	
Stromal karyorrhexis	Present	0	14	.186
	Absent	9	42	
Atypia at extravillous trophoblast	Mild	5	32	> .99
	Marked	4	24	

PGTD, persistent gestational trophoblastic disease.

Discussion

The present study was performed to investigate histopathological parameters commonly used during routine histopathological examination in the differential diagnosis of POC cases, especially

those with early gestational age. Also, we aimed to study the value of using these histopathological criteria for diagnosis of HM specimens by comparing them with the results of p57Kip2 immunohistochemical staining.

In agreement with previous reports, our results showed that examination of the villous shape was statistically significant in differentiation between CHM and PHM cases on one hand and between molar and nonmolar cases on the other hand [16,17]. Most of our studied CHM cases (62%) had a population of distended villi with irregular outline. This was significant in differentiation between POC specimens and in favor of early CHM [15,18]. Our results were in contrast with the results of the study by Ishikawa *et al* [16] who found that enlarged villi with regular round outline was a good marker for the diagnosis of CHM. This disagreement can be explained by the fact that the gestational age of our cases was in the first trimester, and at this early gestational age, villi of CHM don't exhibit the well formed distended villi with regular outline and have more irregular outline.

Cistern formation is a major criterion in HM. Our study showed that cistern formation was found in CHM (96%) more common than in PHM cases (91%). It was significant in differentiation between molar and nonmolar pregnancy. On the other hand, it wasn't significant in differentiation between the CHM and PHM. This is in contrast to other studies that found it significant and in favor of diagnosis of PHM [17,18]. This may be because of the difference in number of studied CHM cases.

We found that the presence of trophoblastic inclusion in the villous core was present in 82% of PHM and in 67% of CHM. This was statistically significant in differentiation between cases of molar and nonmolar pregnancy but was insignificant in differentiation between the CHM and PHM as described in previous reports [16,17,19]. Trophoblastic hyperplasia is an essential requirement for the diagnosis of molar pregnancy [20]. The results of this study demonstrated that 59% of CHM cases had circumferential villous trophoblastic hyperplasia, while 88% of PHM cases exhibited multifocal villous trophoblast hyperplasia. Polar trophoblast hyperplasia was in favor of nonmolar POC diagnosis. It was found to be statistically significant in differentiation between CHM and PHM on one hand and between molar and nonmolar POC on the other hand, in agreement with previous studies [19,20]. On the other hand, our results disagreed with the study of Abdou *et al* [17] on 59 cases of POC specimens and found that trophoblast hyperplasia had no statistically significant value in that differentiation. This marked circumferential villous trophoblast hyperplasia which was found in CHM cases may be related to the absence of p57Kip2 expression by the villous cytotrophoblasts and villous stromal cells in CHM cases which lack the maternal genome. This results in loss of cell cycle control and hence increased trophoblast proliferation.

It is known that villi in CHM have a high level of stromal karyorrhectic debris [21]. In this study, villous stromal karyorrhectic debris wasn't observed in any case diagnosed as PHM or nonmolar

POC. Although the villous stromal karyorrhectic debris was found in 36% of CHM cases, it wasn't statistically significant in differentiation of the three entities; CHM vs PHM or molar vs nonmolar pregnancies. This was in agreement with the finding that stromal karyorrhexis couldn't differentiate between POC cases [17]. This was in disagreement with the results of a study including 113 specimens of POC before 13th week gestation and found that the rate of stromal karyorrhexis was significantly higher in early CHM than in PHM (p<0.001). They proved and confirmed that the frequent karyorrhexis in the villous stroma is a useful histopathologic parameter in the differential diagnosis of CHM from PHM and HA in early gestational age. However, the stromal karyorrhexis couldn't be used as a significant feature in cases showing diffuse hydropic change due to an absence of cellular components in the stroma [18].

The current study showed that atypia (in the form of increased nucleocytoplasmic ratio, hyperchromasia, and pleomorphism) in extravillous trophoblast was mainly of marked degree in 74% of CHM, and of mild degree in 71% of PHM cases. Marked atypia was in favor of CHM diagnosis, while mild atypia was in favor of PHM and HA diagnosis. Marked degree of atypia was statistically significant in differentiation between CHM and PHM specimens, while mild degree of atypia wasn't significant in differentiation between molar and nonmolar POC. It may be a useful histopathologic feature regarding the diagnosis and classification of HM. This was consistent with the observation that trophoblast atypia could differentiate between CHM and PHM where diffuse marked atypia was found in most CHM and focal mild atypia in most PHM [22]. This was in contrast with others who found that atypia in extravillous trophoblast showed no significant value in differentiation between CHM and PHM [17,19].

In our study, seven cases diagnosed histopathologically as PHM were reclassified as CHM after p57Kip2 immunohisto-chemistry (showed no or scattered nuclear staining in villous CT and villous stromal cells). Six of these cases exhibited morphological features similar to PHM on histopathological examination in the form of two populations of villi; small and large distended villi with irregular outline and multifocal villous trophoblast hyperplasia. One case showed two villous populations, circumferential villous trophoblast hyperplasia and trophoblastic inclusion in the core of their villi without stromal karyorrhexis. In these cases, we can consider p57Kip2 as a gold standard for the diagnosis and classification of HM cases. This was similar to previous observations [10].

In a retrospective study done by Landolsi *et al* [2] on 220 specimens of HA, negative p57Kip2 expression was observed in 8 cases with a histopathological diagnosis of PHM, and in one case with a diagnosis of HA. Landolsi *et al*.[2] tried to explain this negative p57Kip2 expression either due to mis-diagnosis of CHM or lack of staining due to loss of antigenicity. They proved the mis-diagnosis

of CHM by genotyping analysis of their discordant nine cases and found absence of their maternal allele [2]. Funkunaga *et al* [7] reported artifactual loss of staining due to loss of antigenicity in four HA and one PHM cases, but the presence of positive internal control in decidual and implantation site trophoblasts in our study excludes this explanation [12].

In the current study, one case histopathologically diagnosed as CHM was inconsistent with the pattern of p57Kip2 immunostaining which showed positive nuclear staining in villous CT and villous stromal cells. This was also found in the results of previous studies in which one case out of 132 CHM cases was found to show positive p57Kip2 immunostaining in their CT and villous stromal cells [2]. This was explained by false-positive immunohistochemistry or a misdiagnosis of PHM or HA. Another possibility is the presence of twin gestation, one of them normal and the other CHM. Furthermore, rare CHMs are biparental in origin and contain both maternal and paternal chromosomal components. They explained it as a mis-diagnosis, as no adequate DNA material was available for genetic study to confirm. In such rare cases, we can't consider p57Kip2 as a gold standard for the diagnosis and the matter will require further studies including molecular techniques using the differences in DNA content.

Concerning the follow-up data, nine cases out of the 65 total cases (14%) developed PGTD. They were diagnosed based on persistence of their symptoms and their serum β-HCG level which didn't come down to the basal level. Eight cases of these were diagnosed as CHM (18% of total CHM). Similar results were found in a study performed by Van Cromvoirt *et al* [23] to identify cases the developed PGTD after CHM; 89 cases (20%) of their total 448 CHM cases developed PGTD and required chemotherapy [24-26].

According to our results, one case of PHM (9% of total PHM) had developed PGTD. But this was inconsistent with the percentage detected in a study done by Wielsma *et al* [24] which detected cases of PHM that developed PGTD; only 6 out of 344 PHM cases (2%) were found to have developed PGTD and were treated successfully by methotrexate chemotherapy. Also, it was inconsistent with the percentage reported by Chen *et al* [3] who found that 2.5%–7.5% of PHM can progress to PGTD. We can attribute this difference between the two studies to the very limited number of the PHM cases studied in the current study. We reviewed these cases histopathologically and didn't find any specific morphological or immunohistochemical features associated with their progression to PGTD. In that regard, the current study was in agreement with the histopathological and immunohistochemical study done by Petts *et al* [25] on 150 cases of molar pregnancy. To achieve this distinction, further studies are required using a larger number of cases and implementing further immunohistochemical markers that may have a benefit.

In conclusion, histopathological diagnosis of molar pregnancy remains problematic especially in early gestational age. The diagnosis requires a constellation of specific histopathological criteria in order not to miss the diagnosis of CHM. p57Kip2 immuno-histochemistry is of great value in the diagnosis of cases that have equivocal morphology by histopathological examination. However, there are no significant features to predict cases that subsequently develop persistent trophoblastic disease. Further immunohistochemical markers should be studied to accurately distinguish PHM from CHM and also to predict patients' outcome.

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Chapter 9

A Novel Genetic Mutation in an Egyptian Patient with Recurrent Biparental Complete Hydatidiform Mole

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Abstract.

Recurrent hydatidiform moles are defined by the occurrence of two or more molar pregnancies in the same patient. A familial recurrent hydatidiform mole (FRHM) is a rare autosomal recessive condition where women have an inherited predisposition to have molar pregnancies. Genotyping demonstrated that they are diploid and biparental.

We report a case of FRHM from Egypt with a history of 6 recurrent complete moles. Sequencing of the *NLPR7* gene revealed a deleterious homozygous base change in exon 2, c.197G>A, which would result in a truncated protein p.W66*. To the best of our knowledge, this mutation has not been described before.

Key words.

Recurrent hydatidiform mole; genetic mutation; NLRP7.

Introduction

Hydatidiform mole is the most common type of gestational trophoblastic disease and may be complete (CHM) or partial. It is a human pregnancy characterized by excessive trophoblastic proliferation and abnormal embryonic development that may be sporadic or recurrent [1]. Recurrent hydatidiform moles (RHMs) are defined by the occurrence of two or more molar pregnancies in the same patient. The frequency of RHMs in England has been reported by Sebire and colleagues to be 1-2 % [2]. However, higher frequencies of RHMs have been reported from the Middle and Far East; where it ranges from 2.5 to 9.4% [3]. Familial recurrent hydatidiform moles (FRHM) is a rare autosomal recessive condition where CHM are diploid and biparental (BiCHM) in contrast to sporadic CHM which are androgenetic (AnCHM) [4] . Eagles and colleagues reported that in the UK 1 in 640 women registered with a CM (0.16%) has this rare condition [4].

Maternal-effect genes are needed in the oocytes to sustain normal embryonic development until the activation of the embryonic genome. Most patients with FRHM, with mutations in both alleles of NLRP7, produce no normal functioning protein necessary to achieve a normal pregnancy [5]. Kou and colleagues have previously identified an intragenic duplication of exon 2-5 in *NLRP7*, which is likely to be the cause of biparental FRHM in 3 unrelated Egyptian families with FRHM [6]. There were no reported mutations in *NLRP7* or *KHDC3L* in women with AnCHM [7]. Akoury and colleagues reported that women with recessive *NLRP7* mutations fail to have normal pregnancies from spontaneous conceptions except for 5 out of 131 reported patients resulted in 6 live births [8].

Because there is no current treatment for RHM, ovum donation appears the best management option for these patients to achieve normal pregnancies [7]. However, ovum donation is not accepted in some communities and is prohibited in Islamic countries.

Methods.

We describe a case of a female with recurrent six CHM who has been managed at Mansoura University Hospital, Egypt.

The available hematoxylin-stained slides of her molar pregnancies were reviewed for confirmation of RHM after being sent to Trophoblastic Disease Screening and Treatment Centre, Imperial College London, Charing Cross Campus, United Kingdom. Genotyping was performed, using paraffin-preserved tissue blocks from the 5th CHM. After confirmation that this was a BiCHM; sequencing of the coding exons of *NLRP7* was performed on genomic DNA of the patient as previously described [9].

Case

A 26 years old otherwise healthy female, G6P0A6, and her 31 years old healthy husband were evaluated after 6 CHM. There was a history of consanguinity between the patients' parents but not between the patient and her husband. There was no history of molar pregnancies among the families of the patient or her husband**.**

Her first pregnancy was misdiagnosed as missed abortion and was surgically evacuated. The patient complained of heavy vaginal bleeding after 20 days of uterine evacuation, ultrasound revealed a residual mass in the uterine cavity measuring 3 x 2 cm, and serum beta human chorionic gonadotropin (β-hCG) was 300,000 mIU/mL. She went through a second curettage, after which the β-hCG sharply declined and normalized. Histopathology of this evacuation product was consistent with CHM.

She conceived five more times; all pregnancies were documented to be CHM by ultrasound and histopathological examination of uterine contents. Spontaneous regression of β-hCG titer after evacuation occurred within 6-8 weeks. The patient received oral contraceptive pills for one year after each pregnancy. The patient did not develop persistent trophoblastic disease after evacuation of any of the six molar pregnancies.

After consenting of the couple; the available hematoxylin-stained slides of the pregnancies were reviewed, all were consistent with CHM. Genotyping of the paraffin – preserved tissue from the fifth pregnancy showed it to be diploid and consistent with a biparental origin. This is consistent with a diagnosis of FRHM. Subsequently genetics testing for mutations in the *NLPR7* gene was performed on genomic DNA from the patient. Sequencing of *NLRP7* revealed a deleterious homozygous base change in exon 2, c.197G>A, which would result in a truncated protein p.W66* (figure 1).

Discussion.

FRHM is a rare condition, in which the affected women have a predisposition to CHM. FRHM can be diagnosed by demonstrating the molar pregnancies are BiCHM in contrast to sporadic CHM which are androgenetic [9]. Approximately 70% of women affected by FRHM are associated with recessive mutations of *NLRP7* [10].

We report a case of FRHM with history of consanguinity but no family history of a similar condition although other authors reported the familial incidence [11,12]. Mutations in NLRP7 have been reported in patients with RHM. Till now, 249 sequence variants are listed; 89 are reported to be pathogenic [13]. These mutations include stop codons, small deletions or insertions, splice

mutations, large deletions or insertions, complex rearrangements, and protein-truncating mutations [14]. Furthermore, Ulker et al. reported a deletion of 60-kb extending from intron 8 of *NLRP7* to intron 11 of *NLRP2* [15]. In the present case a novel deleterious homozygous base change in exon 2, c.197G>A would result in production of a truncated protein p.W66*.This mutation is different from what previously identified by Kou and colleagues Kou and colleagues [6] in 3 unrelated Egyptian families with FRHM.

It was suggested that defective oocytes are responsible for the pathophysiology of RHM. This finding was confirmed when assisted reproductive cycles using donated oocytes in three patients with recessive *NLRP7* mutations resulted in four normal offspring [8]. Unfortunately, at present ovum donation, which is not acceptable in some communities, is probably the most likely way of achieving a normal pregnancy, although a small number of normal pregnancies have been reported in women with FRHM [8]. In addition, Fallahian and colleagues reported a case of two sisters with diploid biparental complete moles. They have a total of six molar pregnancies with no living child. One of them finally conceived of apparently normal female fetus but the placenta showed diffuse molar changes [11]. Spontaneous conceptions in patients with recessive *NLRP7* mutations are rare and usually associated with missense mutations[8], rather than the protein truncating type of mutation in our patient.

The patient did not develop gestational trophoblastic neoplasia (GTN) consistent with other reported studies that the incidence of GTN is not higher in women with FRHM than those with typical sporadic CHM [4,12]. However, further researches may be needed to clarify this point.

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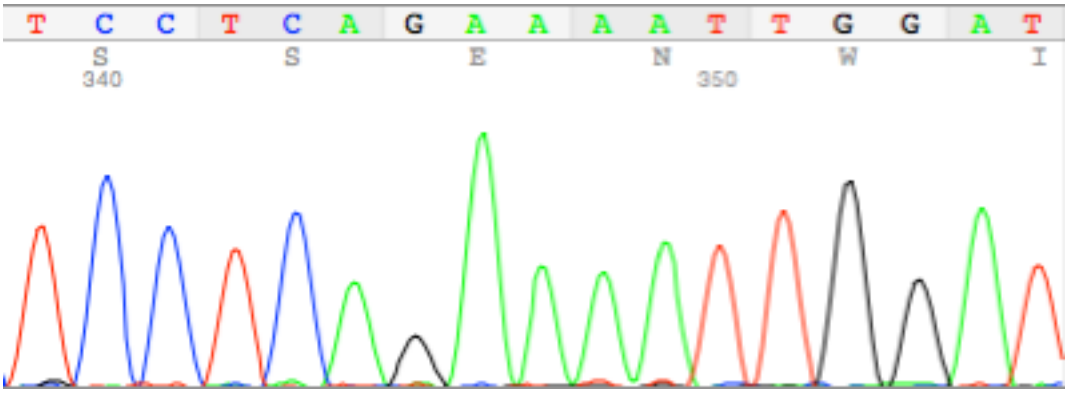
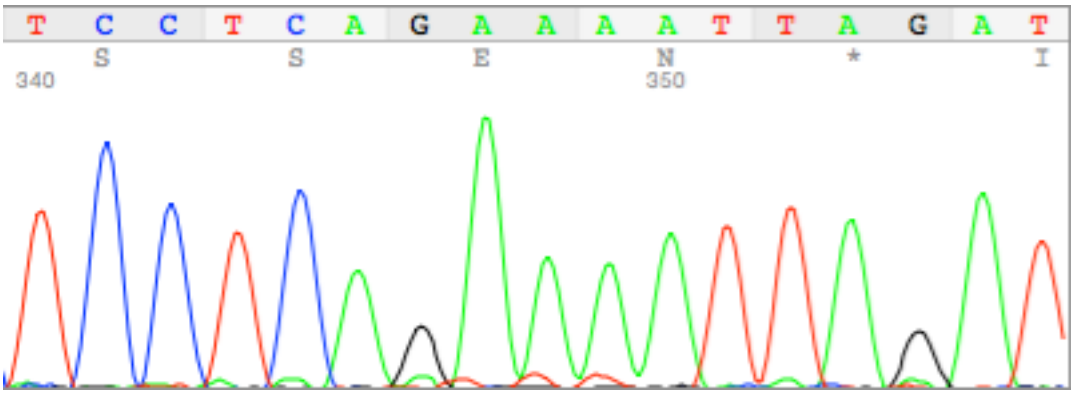
Figure 1: Partial sequence, c.183-198, of *NLRP7* showing a homozygous base change c.197G>A in a patient with FRHM.

Wild Type (normal sequence)



mutated base c.197G>A

Patient Sequence



The mutation results in a change in the amino acid sequence which introduces a stop codon and a truncated protein, p.W66*

Chapter 10

General discussion and future perspectives

10.1 General discussion

In this thesis, we describe our investigation on the epidemiology, clinicopathologic features, and outcome of treatment of GTD at Mansoura University Hospital (chapters 2, 6-8); treatment modalities of women with GTN at 40 years or above (chapter 3); and the role of second curettage in treating low-risk GTN (chapter 4). Subsequently, we discuss the secondary benefits of collaboration between the GTD clinic of Mansoura University, Egypt, and the Erasmus MC Cancer institute, Erasmus University, Rotterdam (chapter 5).

Epidemiology, pathologic features, and outcome of GTD at Mansoura University, Egypt

The incidence of GTD varies globally according to geographic locations. Moreover, the incidence in many non-western countries, including Egypt, is underestimated because of lack of proper diagnostics and proper registration.

We assumed that the incidence of GTD in our locality is underestimated due to reluctance in performing routine histopathologic examination of products of conception. It is often difficult to differentiate retained products of conception from GTD solely on the basis of clinical criteria or imaging; only 40%–60% of CMs and PMs are detected by ultrasound. Contrastingly, 10% of suspected CMs and PMs are non-molar hydropic abortions on histological examination.

To explore the prevalence of GTD in the specimens of pregnancy termination and post-abortive bleeding, we reviewed 640 specimens of the contents of uterine evacuation referred from 2009 to 2014 to the pathology department of Mansoura University. The histopathological diagnoses were correlated to pre-evacuation clinical and ultrasound data, as supplied to the pathologists through referred clinical reports. We found that partial and complete molar pregnancies were histologically diagnosed in 20.6% of the referred cases of miscarriage, which was surprisingly higher than the reported value of nearly 3%. This may be explained by the small sample size of our study. Other explanation is overestimation of molar pregnancy in our study because we included cases referred for histopathological assessment. The third reason is real different incidence of GTD due to differences in geographic distribution.

Undoubtedly, many cases of pregnancy termination in our locality are not subjected to histopathological examination. The most important argument reported was that the clinician was satisfied with his or her clinical and ultrasound diagnosis and felt confirmation superfluous. Another reported reason was the non-availability of pathology laboratories near the clinics in rural areas.

Unfortunately, this is a common problem in the non-Western countries. In case expectant management of miscarriage is followed, the products of conception are often expelled at home; this decreases the chance of specimen examination. In case of persistent or recurrent bleeding, a subsequent curettage follows, and pathological examination is of greater importance in such groups because it can indicate GTN. The authors recommended routine histopathological examination of the products of conception and post-abortive bleeding. If the pathological examination service is not available, the gynecologist should at least inspect the product of miscarriage for the presence of any vesicles and ask the patient for weekly check-up of hCG levels till normalization. To resolve this problem, government should establish pathology laboratories at least in district hospitals. Training courses and national meetings for gynecologists and obstetricians can be good events to announce the importance of routine histopathological examination of the products of conception. Training courses regarding the methods of diagnosis and treatment of GTD are annually organized by the GTD clinic of Mansoura University.

We aimed to explore the incidence and outcome of GTD in Lower Egypt by conducting an observational prospective study. Patients were recruited for 12 months from September 2015 to August 2016 following the establishment of the GTD clinic at Mansoura University and the constitution of a local GTD registry. The GTD clinic at Mansoura University is the first sub-specialized clinic in Egypt. The characteristics, management, and outcome of the patients are reported in *chapter 2*. The hospital-based incidences of molar pregnancy and GTN in Mansoura University Hospital were estimated to be 13.1 and 3.2/1000 live births, respectively. This hospital-based incidence is more than that reported in Taiwan (8.0/1000 deliveries) and Indonesia (9.9/1000 pregnancies). These figures are not the exact figures of incidence of GTD in Delta region, Egypt, because some cases were managed in private clinics. We hope to produce accurate figures in near future when our clinic becomes the only center managing GTD. The reason for relatively high incidence of molar pregnancy in our locality is that Mansoura is a referral center of the entire Lower Egypt. Other reasons are related to socioeconomic factors, such as early age of marriage, pregnancy at maternal age of 40+ years, and less intake of folic acid in diet. Progression to GTN, during follow-up, was found to be 16.1%, which is more than that reported by other authors. The variation in molar progression to GTN in different studies reflects selection bias, but it also reflects different outcomes among different countries. High rate of GTN progression in low-resource countries should be discussed by health care providers in these countries. Moreover, the methods of diagnosis, treatment, and follow-up of molar pregnancies should be improved. Furthermore, some authors have recommended a single-dose prophylactic chemotherapy to patients with criteria of high-risky moles during evacuation to reduce molar progression to GTN.

The ratio of CM to PM in our study was 1.3, which is higher as compared with other reports. This may be explained by the small sample size in our study. However, the high ratio also denotes a more frequent diagnosis of PMs (misdiagnosis) or true higher incidence of PM pregnancies. In many cases, histopathological differentiation between CMs and PMs is difficult, which may necessitate the use of immunohistochemistry showing p57Kip2 or additional genetic studies of the gestational product. It is well-known that p57Kip2 identifies CM by lack of its expression. In *chapter 8*, we aimed to investigate the expression of p57Kip2 in molar pregnancies and their relation to the progression to persistent trophoblastic disease. A total of 65 specimens of the products of conception were reviewed clinically and pathologically, and any progression to persistent GTD was reported. After immunohistochemical staining of p57Kip2, diagnoses of seven cases (10.8%) changed from PM to CM. This is in line with the findings of other authors. Proper differentiation between CM and PM is critical as the rate of progression to GTN is higher in CM than in PM (20% versus 5%), necessitating more attention during follow-up. Furthermore, we found no significant differences in any morphological or immunohistochemical characteristics between cases with or without subsequent development of GTN.

Conclusively, p57Kip2 immunohistochemistry is of great value in the diagnosis of molar cases and implemented in the pathology service in most Western countries. However, being an expensive tool, p57Kip2 immunohistochemistry and genotyping are not routinely available in non-Western countries with limited resources. To solve this problem, both should be performed in a unique central laboratory. Government or non-governmental organizations can be invited to support this sub-specialized laboratory. This central laboratory should perform pathology review for a large area, and it needs to be validated externally according to international standards. Correct diagnoses will improve care for individual patient and guide clinicians to give the best post-surgery follow-up advice.

When such tests are not available and concerns regarding the histopathological diagnosis remains, clinicians may want to advice patients regarding hCG monitoring similar to the protocol for complete molar pregnancy to prevent late diagnosis and treatment of persistent GTD.

During the decision-making for gynecologic cancer, tumor board often faces a challenge between oncological and reproductive outcomes. Most GTN cases occur in young women; thus, fertility-preserving and future procreation are often discussed with patients and their families, particularly in non-Western countries. Reproductive outcomes of fertility-preserving treatment for premalignant and malignant gynecologic diseases are presented in *chapter 6.* Our study included 43 GTN cases. We reported that pregnancy following chemotherapy (43 and 5 cases of GTN and stage 1c ovarian cancer, respectively) resulted in 3 of 48 (6.3%) missed abortions and 1 case of induced abortion.

Adverse pregnancy outcomes were not correlated to the number of chemotherapy cycles but correlated to shorter interval between the previous chemotherapy and subsequent pregnancy. We found that reproductive outcomes after fertility-preserving treatment of GTN were comparable with those of general population, which agreed with other published studies. However, we reported increased rates of cesarean delivery, intra-abdominal adhesions, missed abortion, and congenital fetal malformations. Moreover, one case developed relapse of GTN 1 month after delivery, which necessitated continuing postpartum surveillance of the cured patients.

To conclude, reproductive outcomes after fertility-preserving treatment of GTN were similar to those of the general population. With the ongoing prospective data collection of the GTD clinic at Mansoura University, we aim to further clarify this in future. The long-term effect of chemotherapy on obstetric outcome is still unclear and needs to be evaluated in a large prospective study. In some cases, pregnancy occurred within a short period after chemotherapy, either intended by the patients or after forgetting oral contraceptive pills; thus, this information should be provided while advising regarding pregnancy continuation in women who conceived shortly after cessation of chemotherapy.

RHM is defined as the occurrence of two or more moles. Familial RHM (FRHM) is a rare autosomal recessive condition. *Chapter 9* presents a novel genetic mutation in an Egyptian patient with recurrent biparental CHM. Sequencing of *NLPR7* revealed a deleterious homozygous base change in exon 2, c.197G>A, resulting in truncated protein p.W66*. This test was performed in the laboratory of Trophoblastic Disease Screening and Treatment Centre, Imperial College, London, Charing Cross Campus, United Kingdom; this facility is not available in Egypt. Recently, an international collaboration was initiated between the GTD Clinic of Mansoura University, Egypt, and McGill University, Canada, due to which these tests are now available for women at the GTD Clinic of Mansoura University; this has resulted in diagnosing gene mutations in more Egyptian women. Results of such genetic studies were published within a large number of patients from different countries.

Maternal-effect genes are needed in the oocytes to sustain normal embryonic development until the activation of embryonic genome. Most patients with FRHM, with mutations in both alleles of *NLRP7*, do not produce normally functioning proteins necessary to achieve normal pregnancy. Thus, ovum donation is the best management option for these patients to achieve normal pregnancy. However, ovum donation is prohibited in Islamic and many Eastern countries. This leaves no treatment for RHM. Providing honest and unbiased information to the couple and other involved family members is crucial. The knowledge of carrying such a mutation is distressing for a couple

and other affected females in a family. It may lead to family breakdown when healthy offspring is not guaranteed.

Since some authors have reported sporadic occurrence of normal pregnancy in cases of RHM, the disease should be clearly described to a couple by a specialized gynecologic oncologist with focus on the importance of genetic analysis and very low chance of normal pregnancy. Follow-up should be ensured not only for molar pregnancies but also for subsequent pregnancy outcomes. At the GTD clinic at Mansoura University, we diagnosed over 30 females with recurrent mole during September 2015 to August 2019; to our knowledge, this is one of the largest number reported. Some of these women had a family history of RHM or recurrent miscarriages without pathological diagnosis. Still, some of the patients with RHMs finally had a healthy baby, whereas most did not. We plan to analyze the outcome of these patients to analyze whether we can predict if and when favorable pregnancy outcome can be expected.

Familiar RHM is recessive; thus, in countries with high rates of consanguine marriages, this will be expected to be a noticeable problem. Consanguinity is more prominent in low-resource countries, particularly in traditional countries, than in high-resource countries. Thus, we would like to plea for an internationally funded central laboratory that makes such tests assessable for each couple facing such problems regardless their religion or country. Preferably, the test should be performed using saliva instead of blood because it is easier to send scrapings across oceans than tubes.

Treatment outcome in women with GTN at 40 years old and above

To answer this question, we investigated the outcomes of different treatment strategies for GTN in women aged 40 years and over in a multicenter retrospective study. Treatment of GTN at old age (40 and more years) is poorly studied even though the incidence of molar pregnancy increases in pregnant women at that age. Subsequently, women aged 40 years and over are considered at high risk of developing post-molar GTN. There are no established guidelines for different treatment strategies for GTN at 40 years or over. In *chapter 3*, we presented the experience of five different centers in five countries in four continents (Africa, Asia, Europe, and North America) in the management and outcome of GTN in women at 40 years or more. This study had several limitations, such as its retrospective design, heterogeneity of data reporting from different centers, different local hospital protocols for the management. Moreover, we could not obtain central pathology review of histopathology of the cases. The strength of our study is the considerable size of the cohort with finally 110 patients available for analyses.

In short, we observed that hysterectomy is performed in many cases in the setting of first-line treatment. This is logical considering that in this study; patients completed their families and were aware of their reduced fertility. In low-risk non-metastatic GTN, complete remission was reached in 97.1% of the cases. This high cure rate may be attributed to the adjuvant use of 1–2 courses of MTX after hysterectomy in most participating centers.

Surprisingly, 43% and 56% of women with low-risk and high-risk metastatic disease were initially treated with hysterectomy along with chemotherapy, denoting more frequent use of hysterectomy in the participating centers in this age group. For the high-risk group, complete remission was achieved in 60% of cases who received hysterectomy in combination with chemotherapy compared with 50% of patients who received only chemotherapy. Thus, adjuvant hysterectomy may benefit cautiously selected patients with high-risk GTN, but it should not delay systemic treatment because this remains as the cornerstone of treatment.

Compared with international literature, women with GTN at 40 years old or over were more often categorized to have high risk, higher rates of failure of the MTX/FA regimen in the low-risk group, and higher mortality than GTN in younger patients, all denoting a poorer prognosis of GTN at higher age. This may be attributed to different biological behavior of the tumor. The concept of abnormal biological behavior of GTD at older age is supported by the reported increased incidence of molar progression to GTN (up to 56% in women above 50 years of age). There is also an increased tendency to chemotherapy resistance as reflected in FIGO risk scoring system where 40 or more years of age is scored with a point more than younger age group.

We observed that a relatively large number of patients did not receive the treatment according to international standards. In lower-resource settings, the adverse effects of multi-agent chemotherapy on critically ill patients are feared for; thus, adjusted schedules are needed. In daily practice, step-up schedules are used, such as mono MTX, but they should be followed, e.g., by EMA/CO shortly after the first response because it induces drug resistance and treatment failure and lengthens treatment time unnecessarily. International consensus of experienced clinicians should prepare such protocols. Prospective gathering of clinical data and outcome globally should enable communities to improve knowledge and establish future protocols. Since large international studies are difficult to perform and costly, an easier way is needed to proceed with such projects.

To conclude, for treatment of low-risk non-metastatic disease at old age, hysterectomy with 1–2 courses of single-agent chemotherapy is quite successful and should be discussed with patients as an alternative to hysterectomy or single-agent chemotherapy (MTX/FA or Act-D). A large prospective randomized study can deliver the data to support this. Such randomized study will random low-risk non-metastatic GTN in women at 40 or more years of age into 2 or 3 groups; the

intervention groups will undergo hysterectomy with or without adjuvant single course of MTX, and the control group will receive single-agent chemotherapy with MTX till remission. The primary endpoint will be remission rate (normalization of serum hCG level), whereas secondary endpoints will be the duration of treatment and complications. The GTD clinic at Mansoura University would like to conduct this randomized study in collaboration with other international centers.

Role of second curettage in treatment of low-risk non-metastatic GTN

The impact of second curettage on number of chemotherapy courses, duration of treatment, relapse, and complications during treatment of post-molar GTN is presented in *chapter 4*.

We assumed that second curettage in post-molar GTN produces a "debulking effect" and subsequently reduces the number of chemotherapy courses needed to achieve remission. To study this, we conducted the first randomized controlled study to compare second curettage plus chemotherapy versus chemotherapy alone in patients with post-molar GTN. The study was conducted at Mansoura University Hospital, Egypt, and followed the international guidelines of research conduct. The patients were randomized 1:1 by means of a web-based system, using random block sizes. An intention to treat analyses was performed, including 43 cases in each treatment arm.

We found no significant difference between the two groups. Our results are in line with one retrospective study that concluded that dilatation and curettage of GTN do not affect future chemotherapy requirements; however, the results are also in contrast with two other retrospective and one prospective study. The curative effect of second curettage was not awaited in our study as all patients initially received MTX. Failure of MTX and need of second-line therapy were observed in 15% of the patients; this is in line with previous reports. Since this result was unexpected, we performed an unplanned substandard care analysis to see if results could be compromised by differences between two treatment arms. We did not find any factors that might have influenced these findings. Therefore, we concluded that a second curettage should not be performed in low-risk GTN unless treatment effect (normalization) is awaited for.

In depth, analyzes showed that several factors are at stake that may be of importance in the treatment in other non-Western countries too. We found that treatment cycles with a duration of 17.9 days (comparable in both groups) were longer than the expected 14 days. This was contributed to patient-related factors, such as reluctance to perform blood tests on time and switch to a second-line treatment, difficulties in reaching a clinic, and financial burdens, whereas others were external

factors, such as poor availability of MTX or other second-line drugs in Egypt in certain times due to logistic factors. Therefore, while performing such studies, it should be considered that circumstances for participants should be optimized regardless the allocated treatment arm. To encourage the recruited cases to receive the allocated treatment, the medication (MTX and folinic acid vials) were offered free of charge by the GTD clinic, Mansoura University Hospital. To ensure attendance of follow-up, the patient was contacted over a phone in case of delayed visit.

MTX is available in vials of 50 mg, and in this study, MTX was administered in a fixed dose (50 or 75 mg), allowing subsequent courses to be administered by a nurse in a home setting. The (international) discussion on the best MTX dosing regimen is not yet settled, and some favor a dose related to body weight (1 mg/kg) rather than a fixed dose. Since the two treatment groups were comparable regarding body weight and BMI, the regimen and dosing in this study are unlikely to have affected the primary outcome. However, it is interesting to address this issue in an international study too. We could not trace down the reason for an international community to choose for a fixed dose of 50 mg. In our study, the women had an average weight of 73.5 kg; thus, many might have had a dose >1 mg/kg. It may be necessary to reconsider the strength of fixed dose. In the current era of high numbers of overweight women, it may be needed to adjust the standard dose according to weight, i.e., it can be 50 and 75 mg in women weighing <65 kg and >66 kg, respectively. The industry should be advised to provide ampoules of these two strengths.

Similar problems can be at stake, such as regular hCG level monitoring after molar or post-molar GTN with normalization of hCG level as per protocol. To overcome these problems, the woman was asked to perform and report on urine pregnancy tests. Initially, the tests were performed weekly and then monthly. When the urine test turns positive, the patient was asked to go to a hospital for a serum test and ultrasound scan. Each patient may be granted 6–10 urine testing kits and should be instructed on how to use it. She was asked to bring in the completed tests during each follow-up visit.

Chapter 5 presents the letter to the editor titled “collaboration benefits all”. This reflects the experience of authors from Netherlands and Egypt while conducting the aforementioned randomized study. The collaboration between Western and non-Western countries is essential because it helps in finding proper treatment in many diseases, which are prevalent in low-resource countries. Western countries implement science, knowledge, and advanced technology during proper scientific researches. However, some diseases are rare in Western countries; thus, many years are needed to conduct a study with a proper sample size. Conducting a well-designed RCT according to the international guidelines may be difficult in low-resource settings with high

incidence of these diseases due to poor infrastructure and limited resources. Lack of patients' and physicians' awareness regarding importance of conducting researches is yet another obstacle. WHO encourages this cooperation and helps implement these researches in prevalent diseases in Africa, such as AIDS and cervical cancer. The research collaboration should follow ethics of medical researches, especially ensuring safety, community acceptability, and expected health benefits of enrolled patients.

Other spin offs of this collaboration include the launch of the first GT clinic in Egypt, which now serves as a referral center for patients in Lower Egypt, and the development of a leaflet for patient information in Arabic explaining molar pregnancy of a patient information . We summarized that this collaboration, which was experienced during the conduct of our RCT, helped improve patient management and quality of research at Mansoura University Hospital, Egypt. The authors recommend collaboration in scientific researches between Western and non-Western countries.

As a spin-off of this collaboration and research, the author of this thesis was nominated as the representative of North Africa in the International Society of Study of Trophoblastic Disease (ISSTD).

10.2 Future perspectives

Improvement of the management of GTD at Mansoura University Hospitals, Egypt, necessitates more efforts. The author of this thesis initiated a specialized GTD clinic in 2015, which was the first in Egypt and became the principle referral center in Lower Egypt. A strict registry of all cases of GTD has been established for analyzing accurate data of incidence, prevalence, and outcome. The initiation of Egyptian national registry of GTD is yet another challenge. The registry can be organized by designing a website to include the database of GTD cases from different parts of Egypt. Any interested and dedicated colleague from each university hospital can log in the website with a special password. The basic data to be registered for each case include name (*), date of birth (*), residence, weight, parity, medical history, family history, presentation, hCG level (*), ultrasound criteria, final diagnosis (*), treatment (*), and follow-up details (*); * indicates obligatory fields. After establishing this website, national statistics of incidence and outcome will be easily estimated. Furthermore, this website should have an open access that provides patients and care givers with proper and checked information regarding treatment and prognosis. Since many women in Egypt are illiterate, the information should also be available in local language. Women from the society who suffered from different GTN diagnoses could be asked to participate in this

informative website. A closed part could be used for patients and family members to ask questions regarding difficult issues, such as hereditary forms and resuming procreation and intimacy.

A specialized workshop is organized annually at Mansoura University in collaboration with the ISSTD to increase the knowledge and skills of general gynecologists in the management of GTD.

In future, GTD workshops will also be organized with the help of ISSTD in other countries of North Africa. The gynecologists from these countries will be helped to initiate specialized GTD clinics and start GTD registries, improving local knowledge on GTD and treatment outcomes of such patients.

International collaboration in GTD research is another important perspective. GTD is common in South-East Asia and Middle-East, whereas it is rare in the Western countries. Many countries with high incidence of GTD have limited resources and poor infrastructure for scientific researches; thus, an international collaboration between Western and non-Western countries in conducting GTD research, which follows the international standards and ethical guidelines, will aim at solving health problems that are more prevalent in low-resource countries than—but still present in—Western countries.

Chapter 11

Summary

11.1 English summary.

11.2 Summary in Dutch.

11.3 Arabic summary.

11.1 English summary.

The aim of this thesis was to address studies on gestational trophoblastic disease with emphasis on improving care in Egypt, and second curettage in treatment of low risk gestational trophoblastic neoplasia (GTN) which was not studied before in a randomized study.

In chapter 1, a brief introduction about epidemiology, classification, diagnosis, treatment, and future fertility of gestational trophoblastic disease. In the last section; objectives of the study were presented.

In chapter 2, incidence, demographic criteria, and prognosis of GTD in Mansoura University Hospital were addressed. Incidence and outcome of GTD cases were addressed in an observational prospective study conducted at the GTD Clinic of Mansoura University which serves most of Lower Egypt. The patients were recruited for 12 months from September 2015 to August 2016. We reported 71 clinically diagnosed GTD cases, 62 of them were histologically confirmed, 58 molar (33 CM and 25 PM) in addition to 4 initially presented GTN cases. The incidence of molar pregnancy and GTN at Mansoura University Hospital was estimated to be 13.1 and 3.2 per 1000 live births respectively. The population-based incidence of molar pregnancy and GTN were 0.37 and 0.09 per 1000 live birth respectively. Mean age of the studied cases was 26.22 years. Mean pre-evacuation hCG was 136,170 IU/l. Most of the cases diagnosed accidentally after abnormal sonographic findings (53.2%). The rate of progression of CM and PM to GTN was 24.2% and 8%, respectively and no significant difference between CM and PM regarding hCG level, time to hCG normalization, and progression rate to GTN.

In chapter 3, a retrospective, multicenter study that aimed to investigate the outcome of different treatment strategies in patients with gestational trophoblastic neoplasia (GTN) at 40 years old or above. We analyzed data from 5 referral centers from 5 countries (Egypt, Canada, Ukraine, Saudi Arabia, and Indonesia) during last 5 years. Medical records of 118 women with GTN were retrieved, reviewed and analyzed. Demographic criteria and outcome of different treatment strategies were evaluated.

A total of 118 cases were identified. Mean age was 45.4 years. Of 80 patients with LR non metastatic GTN, 46 women were treated with monochemotherapy. In 34 a hysterectomy with or without (n = 4) chemotherapy was performed. Upfront hysterectomy with or without methotrexate was associated with higher remission rate and shorter treatment duration (P=0.001). In 14 patients with low-risk metastatic GTN, remission rate was 50% for both MTX alone and MTX plus hysterectomy. Two of the 18 high risk patients died before treatment could start and one patient

died during treatment due to distant metastases. Ten HR cases underwent hysterectomy and chemotherapy as first line; 60% achieved remission. Four women were treated with EMA/CO alone; two of them (50%) had incomplete response and needed 2nd line EMA/EP combination.

We concluded that the highest remission rate was seen in the low-risk non-metastatic disease group treated with hysterectomy and subsequent 1-2 courses of methotrexate and hysterectomy with combination chemotherapy in high-risk disease. A large prospective randomized study is recommended to verify these findings.

Chapter 4, presents a randomized-controlled study comparing second curettage plus chemotherapy versus chemotherapy alone. We analyzed 43 patients in each group. The two groups were comparable regarding the basic characteristics. The study concluded that there were no significant difference between the two groups regarding number of chemotherapy courses, methotrexate failure, complications of chemotherapy, and rate of relapse. Moreover, only serum hCG was related to number of chemotherapy courses.

In chapter 5, a letter to the editor which addressed the importance of collaboration in scientific research between developed and developing countries giving the presented RCT in this chapter as an example. The letter clarified that some diseases as GTN and cervical cancer are more common in the developing countries which may have poor resources and infrastructure to conduct proper studies according to the international studies. Other benefits of this collaboration included the launch of the first trophoblastic clinic in Egypt, which now serves as a referral centre for patients in the surrounding area, and the development of a patient information leaflet in Arabic on molar pregnancy.

In chapter 6, we evaluated the obstetric outcome of patients who had undergone fertility preserving treatment for gynecologic premalignant and malignant diseases including gestational trophoblastic neoplasia. The study reviewed the pregnancy course and delivery data of 60 patients (including 43 GTN cases), who conceived after fertility sparing treatment from January 2012 to December 2015. We reported pregnancy complications, abnormalities during labor, neonatal outcome and any recurrence after delivery. We found the mean age of the studied patients was 24.9 years. The preceding lesions included gestational trophoblastic neoplasia (GTN), 43 cases (71.7%) while early ovarian carcinoma (14 cases, 23.3 %) and least CIN III (2 cases, 3.3%) and micro-invasive cervical carcinoma (one case, 1.7%). All cases of GTN were treated with single or multiple agents' chemotherapy. The 3 cases of cervical micro-invasive carcinoma and CIN III were treated with loop electrosurgical excision procedures (LEEP). Cases with stage 1a ovarian cancer were treated with unilateral salpingo-oophorectomy with peritoneal cytology and biopsy of the other ovary. Missed abortion was diagnosed in 4 cases (6.7%). The rate of caesarean delivery was high (70.9%). Intra-

abdominal adhesions were seen in 20.5% of cases. The neonatal outcome was normal in 53 cases while 2 cases (3.6%) of had congenital fetal malformations. The poor neonatal outcome was significantly correlated to number of chemotherapy cycles (P=0.04).

In chapter 7, we studied prevalence of GTD in the referred specimens of uterine evacuation after miscarriage and post-abortive bleeding to the department of Pathology, Mansoura University and private practice settings during the period from 1/1/2009 to 31/3/2014.

Six-hundred referred specimens of contents of uterine evacuation. The mean age of the cases was 26.5 years. The mean gestational age was 10.3 weeks. The commonest clinical diagnosis of the referred cases was missed abortion (51.4%). Molar pregnancy was diagnosed histologically in 103 of 499 referred cases as miscarriage (20.6%). Histopathological examination of specimens of uterine curettage due to post-abortive bleeding revealed GTN in 12 of 27 cases (44.4%). We recommend routine histopathologic examination of uterine contents after pregnancy termination and post-abortive bleeding.

In chapter 8, expression of p57Kip2 in early molar pregnancies and their relations to the progression to GTN was studied. Sixty-five specimens of products of conception were reviewed clinically and pathologically, and any progression to GTN, if present, was noted. Pathological assessment of the archival material included re-cut sections of 5 μm in thickness, routine staining with hematoxylin and eosin and immunohistochemical staining of p57Kip2.Certain histopathological criteria were found to be significant in differentiation between complete hydatidiform mole and partial hydatidiform mole including villous shape and outline, villous trophoblastic hyperplasia, and atypia in extravillous trophoblasts. There were no significant differences in any morphological or immunohistochemical features between cases with or without subsequent development of GTN. We concluded after this study that p57Kip2 immunohistochemistry is of great value in diagnosis of cases that had equivocal morphology. However, there were no significant features to predict cases that subsequently developed GTN.

In chapter 9, we reported a case of FRHM from Egypt with a history of 6 recurrent complete moles. Sequencing of the *NLPR7* gene revealed a deleterious homozygous base change in exon 2, c.197G>A, which would result in a truncated protein p.W66*. To the best of our knowledge, this mutation has not been described before.

Chapter 10 discusses the findings of the presented studies, their impact on clinical practice, and future perspectives.

11.2 Nederlandse samenvatting (Dutch summary).

Dit proefschrift onderzoekt verschillende aspecten van trofoblast ziekten, met de nadruk op het evalueren en verbeteren van de zorg in Egypte, en op het effect van een tweede curettage op het aantal chemotherapie kuren in het geval van laag-risico, persisterende trofoblast ziekte (GTN).

Hoofdstuk 1, bestaat uit een korte introductie over epidemiologie, classificatie, diagnose, behandeling, en toekomstige vruchtbaarheid van GTN. In het laatste gedeelte worden de doelstellingen van het proefschrift en de onderlinge samenhang van de verschillende studies gepresenteerd.

Hoofdstuk 2, beschrijft de incidentie, demografische criteria en prognose van GTD in het Mansoura University Clinic. Dit betreft een prospectieve, observationele studie van de patiënten behandeld in de gespecialiseerde trofoblastziekte kliniek (GTD kliniek) van Mansoura Universiteit. Patiënten uit het gebied Neder-Egypte worden hiernaar toe verwezen. De patiënten werden gerekruteerd van september 2015 tot augustus 2016. We rapporteerden 71 klinisch gediagnosticeerde GTD-casus, waarvan 62 histologisch bevestigd, 58 mola en 4 andere GTN-casus. De incidentie van mola zwangerschap en GTN in Mansoura University Clinic werd geschat op respectievelijk 13,1 en 3,2 per 1.000 levendgeborenen. De incidentie in de populatie van mola en GTN zijn geschat op respectievelijk 0,37 en 0,09 per 1.000 levendgeborenen. De gemiddelde leeftijd was 26,2 jaar. Gemiddelde pre-evacuatie hCG was 136.170 IE/l. De meeste gevallen werden gediagnosticeerd na abnormale echografische bevindingen (53,2%). De mate van progressie van CM en PM naar GTN was respectievelijk 24,2% en 8%, er was geen significant verschil tussen CM en PM met betrekking tot hCG-niveau, tijd tot hCG-normalisatie en progressiesnelheid naar GTN.

Hoofdstuk 3, betreft een retrospectieve studie naar de uitkomst van verschillende behandelingsstrategieën bij patiënten met GTN van 40 jaar en ouder. We hebben de afgelopen 5 jaar gegevens van 5 referentiecentra uit 5 landen (Egypte, Canada, Oekraïne, Saoedi-Arabië, en Indonesië) geanalyseerd. Medische dossiers van 118 vrouwen met GTN werden opgehaald, beoordeeld en geanalyseerd. Demografische criteria en uitkomsten van verschillende behandelingsstrategieën werden geëvalueerd. De gemiddelde leeftijd was 45,4 jaar. Van 80 patiënten met Laag risico niet-gemetastaseerd GTN, werden 46 vrouwen behandeld met monochemotherapie. Bij 34 werd een hysterectomie met of zonder (n = 4) chemotherapie uitgevoerd. Deze behandeling was geassocieerd met een hoger remissiepercentage en een

kortere behandelingsduur (P=0,001). Bij 14 patiënten met laag risico gemetastaseerd GTN was het remissiepercentage 50% voor zowel Methotrexaat (MTX) alleen, als voor MTX plus hysterectomie. Twee van de 18 patiënten met een hoog risico stierven voordat de behandeling kon beginnen en één patiënt stierf tijdens de behandeling als gevolg van metastasen op afstand. Tien hoog risico casus ondergingen hysterectomie en chemotherapie als eerste lijn; 60% bereikte remissie. Wij concludeerden dat het hoogste remissiepercentage werd waargenomen in de niet-metastatische ziektegroep met een laag risico die werd behandeld met hysterectomie en de daaropvolgende 1-2 kuren. Een groot prospectief gerandomiseerde studie wordt aanbevolen om de waarde van de hysterectomie in de hoog risico groep en in de groep met laag risico en gemetastaseerde ziekte te onderzoeken, omdat is gebleken dat ook in deze groepen onverwachts vaak in de eerste behandellijn voor een hysterectomie wordt gekozen.

Hoofdstuk 4 presenteert een gerandomiseerde gecontroleerde studie waarin een tweede curettage plus chemotherapie versus de chemotherapie alleen wordt vergeleken in vrouwen met persisterende trofoblast ziekte na een mola zwangerschap. We hebben 43 patiënten geanalyseerd in elke groep. De twee groepen waren vergelijkbaar wat betreft de basiskenmerken. De studie concludeerde dat er geen significant verschil was tussen de twee groepen met betrekking tot het aantal chemotherapiekuren, methotrexaat falen, complicaties van de chemotherapie, en de mate van terugval. Bovendien was alleen serum hCG gerelateerd aan het aantal chemotherapiekuren.

Hoofdstuk 5, weerspiegelt een brief aan de redacteur waarin het belang van samenwerking in wetenschappelijk onderzoek tussen een Westers en niet- Westers land. Hierbij werden de lessen geleerd uit het in hoofdstuk 4 gepresenteerde onderzoek gedeeld. Wij schreven dat sommige ziekten zoals GTN en baarmoederhalskanker vaker voorkomen in de landen die over minder of onvoldoende middelen en infrastructuur beschikken om volgens de internationale richtlijnen studies correct uit te voeren. Andere voordelen van deze samenwerking werden besproken, zoals de lancering van de eerste trofoblast kliniek in Egypte, die nu dient als een referentiecentrum voor patiënten in de omgeving, en de ontwikkeling van een folder met patiëntinformatie in het Arabisch over molaire zwangerschap.

In hoofdstuk 6, hebben we de verloskundige uitkomst van patiënten geëvalueerd die een vruchtbaarheid sparende behandeling hebben ondergaan voor gynaecologische premaligne en

kwaadaardige ziekten met inbegrip van GTN. De studie behelst het zwangerschapsverloop en bevallingsgegevens van 60 patiënten (inclusief 43 GTN-gevallen), die zwanger werden na een vruchtbaarheid sparende behandeling tussen januari 2012 en december 2015. We rapporteerden zwangerschapscomplicaties, afwijkingen tijdens de bevalling, neonatale uitkomst en het aantal zwangerschappen. De gemiddelde leeftijd was 24,9 jaar. De voorgaande diagnoses waren GTN (n = 43, 71,7%) en laag stadium ovariumcarcinoom (n = 14, 23,3%). Alle GTN-cases werden behandeld met chemotherapie. Drie casus met micro-invasief cervixcarcinoom of CIN III ondergingen een lis-excisie van de transformatie zone. Stadium 1a eierstokkanker werden behandeld met unilaterale salpingo-oophorectomie met peritoneale cytologie en biopsie van de andere eierstok. Vier vrouwen (6,7%) kregen een spontane miskraam. Bij 70,9% werd een keizersnede verricht, bij 20.5% werd daarbij verklevingen gezien. De neonatale uitkomst was in het merendeel normaal (n = 53), in 2 gevallen (3,6%) werden congenitale afwijkingen gevonden.

In hoofdstuk 7 hebben we de prevalentie van GTD bestudeerd in de histologische preparaten van baarmoederevacuaties na een miskraam en post-abortieve bloedingen gepresenteerd aan de afdeling Pathologie van de Mansoura University Clinic en privépraktijken in de periode van 1/1/2009 tot 31/3/2014.

In totaal werden 600 miskraam curettage preparaten onderzocht. De gemiddelde leeftijd was 26,5 jaar. De gemiddelde zwangerschapsduur was 10,3 weken. De meest voorkomende klinische diagnose van de verwezen gevallen was gemiste abortus (51,4%). Mola zwangerschap werd gediagnosticeerd in 103 van de 499 als miskraam verwezen casus (20,6%). Histopathologisch onderzoek na post-abortieve bloeding onthulde GTN in 12 van de 27 gevallen (44,4%). Wij adviseren routinematig histopathologisch onderzoek van de baarmoederinhoud na curettage in de zwangerschap en post-abortieve bloeding.

In hoofdstuk 8 werd de expressie van p57Kip2 in vroege molaire zwangerschappen en hun relatie tot de progressie naar GTN bestudeerd. Vijfenzestig histologische preparaten van conceptieproducten werden klinisch en pathologisch beoordeeld, en elke progressie naar GTN, werd genoteerd. Pathologische beoordeling van het archiefmateriaal omvatte opnieuw gesneden secties van 5 μm dik, routinekleuring met hematoxyline en eosine en immunohistochemische kleuring van p57Kip2. Bepaalde histopathologische criteria bleken significant te zijn in differentiaties tussen complete hydatidiforme molazwangerschap en partiele hydatidiforme molazwangerschap, waaronder een villeuze vorm, villeus trofoblastische hyperplasie en atypie

in extravilleuse trofoblast. Er waren geen significante verschillen in morfologische of immunohistochemische kenmerken tussen gevallen die zich wel of niet ontwikkelde tot GTN. We concludeerden na deze studie dat p57Kip2-immunohistochemie van grote waarde is bij de diagnose in geval van een niet goed te duiden morfologie. Er waren echter geen specifieke kenmerken die de ontwikkeling tot GTN voorspelden.

In hoofdstuk 9, we rapporteerden een geval van FRHM uit Egypte met een geschiedenis van 6 terugkerende complete mola's. Sequencing van het NLPR7-gen onthulde een schadelijke homozygote baseverandering in exon 2, c.197G> A, wat zou resulteren in een afgeknot eiwit p.W66*. Voor zover wij weten, werd deze mutatie nog niet eerder beschreven.

Hoofdstuk 10 bespreekt de bevindingen van de gepresenteerde studies, hun impact op de klinische praktijk en toekomstperspectieven.

11.3 Arabic summary

الملخص العربى

هذه الرسالة تهدف إلى إلقاء الضوء على خصائص مرض أورام المشيمة الخبيثة وطرق علاجها مع تركيز خاص على دور إعادة عملية كحت الرحم فى العلاج .

فى الباب الأول من هذا العمل : تم عرض مقدمة عن الحمل العنقودى وأنواعه وطرق التشخيص والعلاج . وفى نهاية هذا الجزء تم عرض أهداف هذه الدراسة .

الباب الثانى يعرض أول دراسة مستقبلية تمت فى عيادة الحمل العنقودى بجامعة المنصورة وقد استمرت الدراسة لمدة عام (من سبتمر 2015 حتى أغسطس 2016) حيث شملت 71 مريضة بالحمل العنقودى وأورام المشيمة . وقد وجد أن نسبة حدوث الحمل العنقودى وأورام المشيمة فى جامعة المنصورة هى 13,1و 3,2 لكل 1000 مولود بالتوالى . ونسبة تحول الحمل العنقودى الكلى والجزئى 24,2% و 8% على التوالى .

وفى الباب الثالث تم عمل دراسة راجعه بمراكز طبية متعددة لدراسة نتائج أساليب العلاج المختلفة للمريضات ذات أورام المشيمة البالغات من العمر أربعون عاما أو أكثر فى خمس دول مختلفة وهم (مصر , كندا , أوكرانيا , المملكة العربية السعودية و أندونسيا) . وقد وجد أن متوسط عمر المريضات 45,5 ومتوسط هرمون الحمل الرقمى هو 1390,5 وحدة ومعظم هؤلاء المريضات كانوا فى المرحلة الأولى . كما ان إنتشار المرض بالجسم تم تشخيصه فى 28 حالة وكانت الرئة أكثر الاماكن إنتشارا . وقد تم علاج بعض المريضات بالعلاج الكيماوى وبعضهم بإستئصال الرحم ووجد أن إستئصال الرحم مع عقار الميثوتريكسات للمريضات الأقل خطورة و إستئصال الرحم مع العلاج الكيماوى المركب للمريضات الأكثر خطورة أعطى نسبة نجاح أعلى من العلاج الكيماوى فقط .

وفى الباب الرابع دراسة عشوائية للمقارنة بين إعادة كحت الرحم بالإضافه إلى عقار الميثوتريكسات مع الميثوتريكسات فقط . وشملت الدراسة 43 مريضة فى كل مجموعة ووجد أنه ليس هناك فرق إحصائى بين كلتا المجموعتين فى عدد مرات العلاج الكيماوى أو فشله أو حدوث المضاعفات .

والباب الخامس يوضح أهمية التعاون فى البحث العلمى بين الدول النامية والدول المتقدمة تمت دراستها فى مقال علمى والمعروف أن أمراض مثل أورام الحمل العنقودى والمشيمة وسرطان عنق الرحم أكثر شيوعا فى الدول النامية حسب الدراسات الدولية مما يجعل أولوية عمل الأبحاث بهذه الدول . كما يوضح أهمية وجود مركز متخصص لعلاج الحمل العنقودى وأورام المشيمة بمصر وغيرها من الدول النامية مما يزيد من طريقة علاج المريضات .

وفى الباب السادس تم تقييم نواتج الحمل المختلفة للمريضات ذواتى الأورام النسائية الخبيثة بأسلوب العلاج للحفاظ على الخصوبة . وشملت الدراسة 60 مريضة (43 منهن تعانين من أورام للمشيمة) فى الفتره من يناير 2012 حتى ديسمبر 2015. وتم

تسجيل نواتج ومضاعفات الحمل والولادة وحالة المواليد وتكرار المرض بعد الولادة . ووجد أن هناك زياده فى نسب الولادات القيصرية والتصاقات البطن الداخلية أما بالنسبة إالى نتائج المواليد فكانت مساوية للسيدات الحوامل السليمة .

وفى الباب السابع تم دراسة مدى شيوع أورام المشيمة فى عينات تفريغ الرحم مابعد الإجهاض أو نزيف مابعد الإجهاض بقسم الباثولوجى – جامعة المنصورة – مصر والمراكز الطبية الخاصة فى الفتره من 2009/1/1 حتى 2014/3/31 . وتم فحص 600 عينة لمحتويات الرحم ووجد أن الإجهاض المركون هو الأكثر تشخيصا وأن نسبة الحمل العنقودى كانت 20% . ولذلك يوصى بدراسة الأنسجة الخلوية لمحتويات الرحم بعد إنهاء الحمل أوفى حالات نزيف مابعد الإجهاض .

وبالباب الثامن دراسة العلاقة بين وجود الطفرة الجينية P57Kip2 فى الحمل العنقودى المبكر وتطوره إلى حمل عنقودى مستمر . وتمت الدراسة على 65 عينة ووجد أن دراسة هذا الجين ذات أهمية كبيرة فى التشخيص لكنه ليس له أى أهمية فى توقع مدى تطور الحالة إلى أورام الحمل العنقودى المستمر .

وبالباب التاسع تم ذكر تاثير الطفرات الجينية على مريضات الحمل العنقودى المتكرر . وقد قمنا بوصف حالة تعانى من حمل عنقودى متكرر 6 مرات وتم تحليل الحمض النووى لها ووجد أنها تعانى من طفرة جينية فى الجين (NLRP7) وجدير بالذكر أن هذه الطفرة لم يتم تسجيلها دوليا من قبل .

Chapter 12

Appendices

1. Avoid making a mountain out of an invasive hydatidiform mole: do a pregnancy test! A case report.

Published as: R Hemida, HC van Doorn. Avoid making a mountain out of an invasive hydatidiform mole: do a pregnancy test! The Lancet 2019; 394 (10194), e2.

Case report.

A 28 years old female patient was referred to the Trophoblastic Clinic of our hospital after her postoperative histopathology following hysterectomy revealed an "invasive mole". The patient had normal menstrual cycles of 30 days with 5 days bleeding, but at the age of 26 years, her bleeding became irregular, prolonged and much heavier lasting 15-20 days with no prior missed periods. The patient had no history of contraception, hormonal therapy, or other medications. After three months of irregular bleeding, the patient visited her local gynaecologist who reported no definite uterine or cervical lesions and prescribed non-specific haemostatics. The patient's hemoglobin was10 gm/dl but, neither urine nor serum hCG was checked.

For continuous bleeding problems, the patient underwent dilatation and curettage of uterus with endometrial biopsy that revealed "disordered proliferative endometrium". The patient was advised to receive combined oral contraceptive pills that only decreased the uterine bleeding. Six month later, a repeated transvaginal ultrasound scan revealed focal anterior myometrial thickening with increased Doppler activity and the lesion was interpreted as "adenomyosis" (figure). Hysteroscopic endometrial resection was advised but severe bleeding occurred during the procedure and was stopped. Thereafter, profound bleeding persisted, her general condition deteriorated, and haemoglobin dropped to 6 gm/dl. After stabilizing with blood transfusion, a life-saving uncomplicated abdominal hysterectomy was performed. Histopathological examination of uterus revealed invasive mole without evidence of uterine perforation. Serum hCG was 550 IU/L and she was diagnosed with low-risk gestational trophoblastic neoplasia. Remission occurred after 2 courses of methotrexate.

The cause of persistent uterine bleeding was not suspected as ultrasound image might be similar to that of other lesions as adenomyosis. Endometrial biopsy did not detect the lesion which existed in the deep myometrium. This case scenario shows that hCG test should not be neglected during initial assessment of abnormal uterine bleeding, as a trophoblastic tumour is easily cured often with single agent methotrexate without the need to perform a hysterectomy.

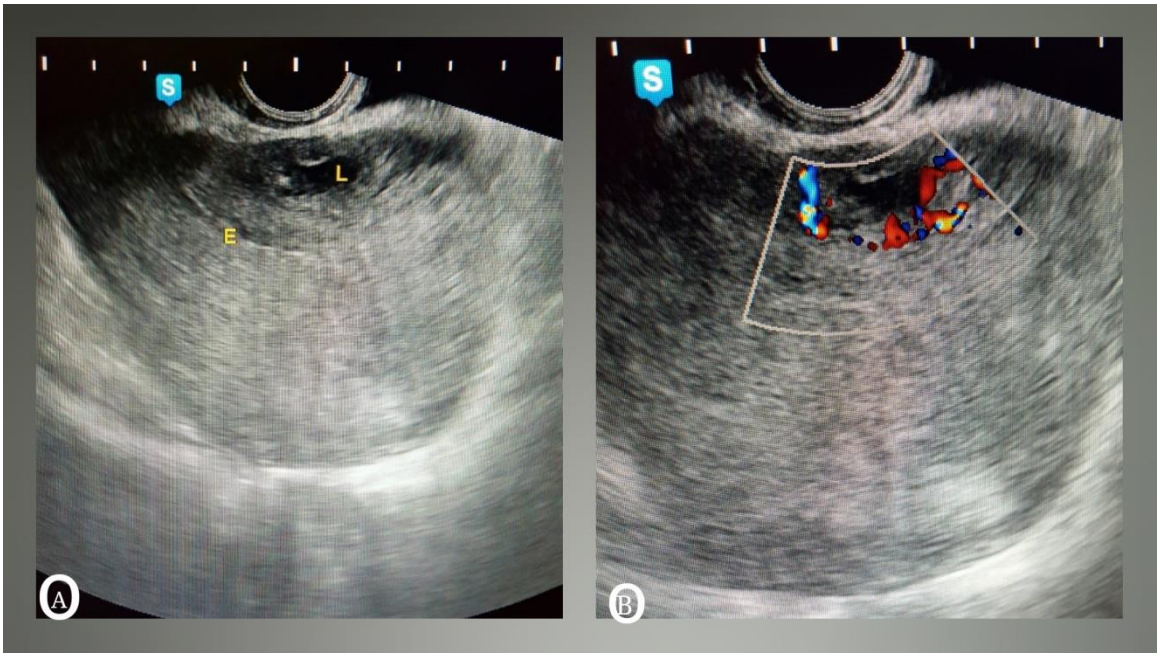


Figure: (A): Transvaginal ultrasound, E: endometrium. L: lesion; a focal heterogeneous area of anterior myometrium 3x2 cm. (B): Colour Doppler shows increased flow.

12.2 Patient information leaflet in Arabic:

يحتوي هذا الكتيب عن معلومات عن تشخيص وعلاج و متابعة الحمل العنقودي وأورام المشيمة .

يمكن علاج هذا المرض بنجاح كبير عند اكتشاف المرض في بدايته وعندما تكون المريضة وأسرتها والأطباء يتبعون ارشادات العلاج الدولية.وعند تأخر العلاج أو توقفه قبل الأوان يمكن أن يصبح هذا المرض صعب جدا للعلاج، وربما تفقد المرأة القدرة علي الحصول علي مزيد من الحمل، أو قد لا يمكن الشفاء منه،

لذلك نطلب منك قراءة هذه المعلومات بعناية . يمكنك أن تسألي أي أسئلة متبقية للأطباء

<u>عن الحمل العنقودي :</u>

يحدث الحمل العنقودي بسبب اضطراب في تلقيح البويضة بواسطة الحيوان المنوي، ويؤدي إلي نمو خلايا غير طبيعية أو مجموعات من حويصلات المياه تملأ الرحم. معظم حالات الحمل العنقودي هي حميدة (غير سرطانية). وإما أن يكون كامل أو جزئي. وهو مرض نادر الحدوث (يصيب امرأة حامل من كل 1000 امرأة)

و يمكن أن تنتشر خارج الرحم عند بعض النساء، ولكن لا تزال قابلة للعلاج. تحتاج النساء للمتابعة الدقيقة لاكتشاف المرض المتبقي وعلاج هذا علي الفور

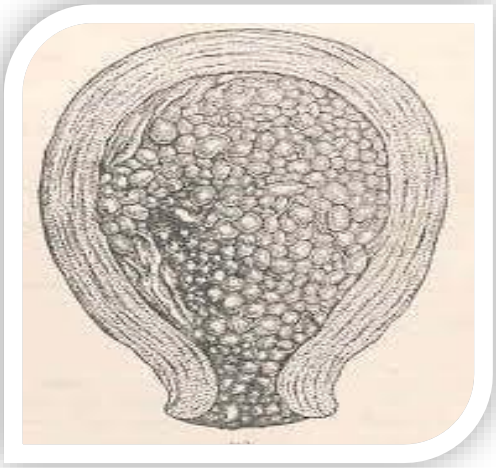


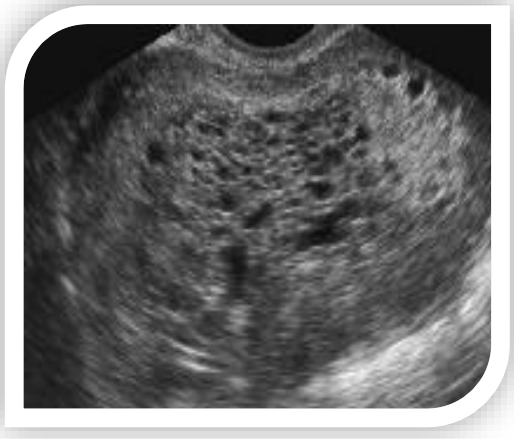
Diagram of uterus containing a complete mole

الحمل العنقودي الكامل والجزئي

إذا كان لديك الحمل العنقودي الكامل، لا تتكون أجزاء من الطفل (الأنسجة الجنينية). يوجد فقط أنسجة الحمل العنقودي في الرحم. إذا كان لديك الحمل العنقودي الجزئي قد يكون هناك بعض أنسجة للجنين في الرحم، جنبا إلي جنب مع أنسجة الحمل العنقودي. وبالرغم كم ذلك فالتصوير بالموجات فوق الصوتية قد تبدو كأنها الجنين

<u>علاج ومتابعة الحمل العنقودي</u>

عادة ما يتم إزالة الأنسجة العنقودية بعملية كحت للرحم، وسيتم ذلك وأنت تحت التخدير. الطبيب سوف يوجه هذا العلاج مع الموجات فوق الصوتية بعد ذلك، في نحو 10 إلي 15 من أصل 100 امرأة تظل بعض أنسجة الحمل العنقودي في الأنسجة العميقة من الرحم أو أجزاء أخري من الجسم. وهذا ما يسمي أورام المشيمة المستمرة و يحتاج إلي علاج للكشف عن أورام المشيمة المستمرة يتم اختبار هرمون الحمل في الدم. هرمون الحمل هو هرمون ينتجه الحمل الطبيعي ولكن أيضا من قبل الحمل العنقودي واورام المشيمة. مستوي هذا الهرمون في الدم يمثل كمية الأنسجة غير الطبيعية، وبالتالي يستخدم لمعرفة ما غذا كان تم إزالة جميع الأنسجة



Ultrasound picture of complete mole

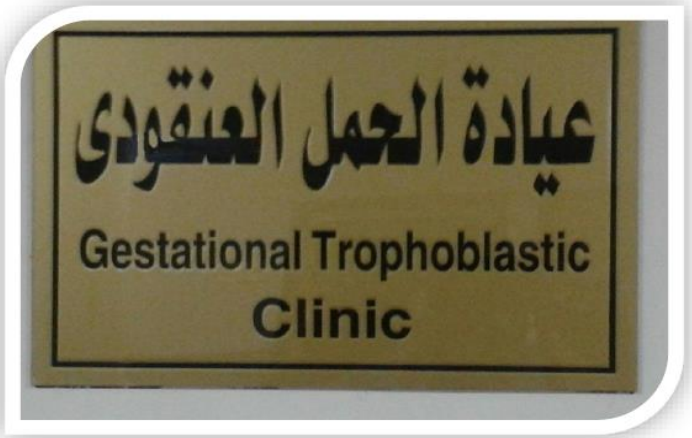
إذا كان مستوى هرمون الحمل الخاص بك يعود إلي طبيعته قريبا بعد إزالة الحمل العنقودي، فأنت لا تحتاجي إلي مزيد من العلاج. في معظم النساء مستوي هرمون الحمل يختفي تقريبا في غصون 4 إلي 6 أسابيع من إزالة الحمل العنقودي. بمجرد اختفاء الحمل العنقودي لا يمكن أن ينتج هرمون الحمل.

بعد عودة التحاليل للمستويات الطبيعية يطلب منك اختبار شهريا لهرمون الحمل في الدم لمدة 6 أشهر. و لهذا يجب الامتناع عن الحمل لهذا الوقت (6 أشهر) للتأكد من شفاك التام ولذلك ننصح باستخدام حبوب منع الحمل لهذه الفترة

متي تكون المتابعة غير طبيعية ؟

عند حدوث أي من الاتي بعد تشخيص أورام المشيمة يطلب المزيد من التحاليل والعلاج :

1- مستوي هرمون الحمل الخاص بك يبقي علي حالة في 4 قياسات علي مدي 3 أسابيع أو أكثر
2- يرتفع مستوى هرمون الحمل الخاص بك في 2 قياسات في صف واحد، علي مدى فترة أسبوعين على الأقل.
3- مستوى هرمون الحمل الخاص بك يظل مرتفعا لمدة 6 أشهر أو أكثر، حتى لو بدأ يقل.
4- وجود دليل علي أورام المشيمة في عينات الانسجة التي أخذت أثناء ازالة الحمل العنقودي.
5- استمرار نزيف مهبلي مع ارتفاع مستوى هرمون الحمل.



أورام المشيمة المستمرة.

استمرار اورام المشيمة يحدث بعد ازالة الحمل العنقودي لكن بعض الانسجة متبقية وتنمو مكونة ورم جديد، اذا لم يتم علاجها ستنمو سريعا مكونة عددا من المشاكل الطبية، واحيانا خطيرة جدا. من الممكن علاج معظم النساء من أورام المشيمة بشكل فعال بالعلاج الكيماوي لكن علي المريضات والاطباء الالتزام بالبروتوكول للعلاج باسرع وقت ممكن حتى يمكن التخطيط لحمل جديد.

خطة العلاج

بعد تشخيصك بأورام المشيمة، يتم تحديد مدى او مرحلة تطور المرض ووجود بعض عوامل الخطورة التي تؤدي الي اللجوء للعلاج الكيماوي وأي الادوية المناسبة لك

اختبارات مرحلة المرض

يقوم الاطباء بعمل التحاليل والفحوصات اللازمة للحصول علي معلومات تفيد في تحديد مدى انتشار المرض وهذا يسمى بـ " تحديد مرحلة الورم " من المهم معرفة اذا ما كانت أورام المشيمة في الرحم فقط أو انتشرت إلي أجزاء أخري من الجسم، تحديد مرحلة الورم مهم لمعرفة العلاج بناء علي مرحلة الورم

يطلق علي نظام تحديدمرحلة أورام المشيمة اسم , "FIGO" يقوم هذا النظام بتحديد مرحلة الورم وعوامل الخطورة ليجد العلاج المناسب، يتم استخدام ترقيم لمرحلو المرض وعوامل الخطورة، من الممكن أن تسألي طبيبك بعد اجراء التحاليل علي مرحلة الورم لديك

<u>عوامل الخطورة</u>

بعد تشخيصك بأورام المشيمة يبحث طبيبك عن عوامل الخطورة مثل : سنك، مستوى هرمون الحمل، وعوامل أخري...

يصنف الاطباء النساء إلي مجموعتين عالية الخطورة وأقل خطورة للبت في العلاج الكيماوي، معظم النساء التي تحتاج لعلاج بعد الحمل العنقودي تكون علي درجة منخفضة الخطورة وتعطي علاج كيماوي واحد

<u>علاج أورام المشيمة " ذات معدل خطورة أقل "</u>

يستخدم العلاج الكيميائي لقتل خلايا السرطان. عن طريق عرقلة نموها, العلاج الكيميائي يدور في مجري الدم في جميع أنحاء الجسم. إذا كان لديك ورم من النوع الاقل خطورة فالعلاج هو دواء " الميثوتريكسيت " و يعطي حقن عضل يوم بعد يوم في واحدة من عضلات الساق ..

- قد تضطري إلي البقاء في المستشفى الأسبوع الأول من العلاج. ومن الممكن أن تستكملي باقي العلاج في البيت مع متابعة في العيادات الخارجية
- يتم اعطاؤك 4 حقن ثم 7 ايام بدون أي علاج قبل بداية دورة جديدة من العلاج. بعد 24 ساعة من أخذ الحقنة يتم اعطاؤك دواء " حمض الفوليك " للحد من الآثار الجانبية للميثوتريكسيت. في كثير من النساء يتم التخلص نهائيا من أورام المشيمة بالعلاج الكيماوي ولا تحتاج إلي أي علاج أخر
- عدد دورات العلاج الكيماوي يعتمد علي مستوي هرمون الحمل في الدم. أثناء العلاج يتم قياس مستوى هرمون الحمل في الدم باستمرار. سيستمر العلاج الكيماوي حتي يختفي هرمون الحمل نهائيا وتصبح التحاليل طبيعية. وعندها يصبح جسمك خاليا تمام من أي خلايا لأورام المشيمة بعدها يتم اعطاؤك العلاج الكيماوي من 4 لـ 6 أسابيع.

حقن الميثوتريكسيت تسبب بعض الآثار الجانبية الطفيفة كالآتي:

1- تغير في طعم التذوق
2- قرح سطحية بالفم واللسان
3- الاسهال
4- آلام طفيفة بالبطن والصدر

المتابعة بعد العلاج من أورام المشيمة

بعد علاجك سيقوم طبيبك بقياس مستوي هرمون الحمل في الدم لمدة 12 شهر . لذلك يجب الامتناع عن الحمل في هذه الفترة عن طريق استخدام حبوب منع الحمل للتأكد من شفاك التام.

لا يتأثر الحمل الجديد بعد علاجك من الحمل العنقودي أو أورام المشيمة. إذا عادت أورام المشيمة مرة ثانية بعد العلاج الكيماوي ستحتاجي مزيدا من العلاج الكيماوي يعطي عن طريق الوريد في مدة قصيرة وذلك بناء علي البروتوكولات الدولية.

12.3 PhD PORTFOLIO

Name of PhD Student:	Reda Hemida
Erasmus MC Department :	Gynecologic Oncology
PhD Period:	November 2012 - February 2019
Promotor:	Prof. dr. Curt W Burger
Co-promotor:	Dr. Helena C van Doorn

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Training Courses and Workshops

	Year	ECT
A. General courses.		
1. **Research Ethics Online Training course**, designed and produced by the World Health Organization (WHO).	2017	0.5
2. 5th International Training Course of **Scientific Writing and Publication**. Mansoura Urology and Nephrology Center. Mansoura, Egypt.	2014	1.0
3. **Design and Review of scientific research**. Mansoura University Development Center.	2016	0.4
4. **Statistical Analysis Skills**. Mansoura University Development Center, Mansoura.	2016	0.4
5. **Design and preparation of electronic tests**, Mansoura University Development Center	2016	0.4
6. Springer Journal Author Academy online course: **"Writing Your Manuscript"** www.Academy.Springer.com**.**	2015	0.3
7. Education program of American College of Obstetrics and Gynecology **"ACOG clinical perspectives in Obstetrics and Gynecology"**.	2015	2.0
B. Specific training courses in Gynecologic Oncology:		
1-Training in Gynecologic Oncology unit, **Erasmus MC, Rotterdam, The Netherlands**: (4 weeks)	2012	6.0
2- **Masterclass in Gynecologic Oncology**, Madrid-Spain.	2014	0.5

3- The Gynecologic Oncology and Surface Malignancy Workshop. Mansoura Oncology Center. Mansoura.	2017	0.5

Organizing training courses in Gynecologic Oncology:

1- The 1st, 2nd, and 3rd courses of "Early detection of Gynecologic cancers. Mansoura University- Egypt . (0.5 X 6)	2013-2018	3.0
2 – The First ESGO endorsed Gynecologic Oncology Workshop in Mansoura, Egypt.	2015	0.5
3- The 1st workshop on Gestational trophoblastic tumors: Diagnosis, treatment, and follow up. Faculty of Medicine, Mansoura University.	2017	0.4
4-The 2nd international workshop in collaboration with ISSTD: Updates in management of trophoblastic tumors, Mansoura University.	2018	0.4

Attended Conferences

-A speaker in an international conferences:

1. XIX World Congress of Gestational Trophoblastic diseases, Amsterdam, the Netherlands.	2017	2.0
2. 10th Breast and Gynecologic Immunotherapy Cancer Conference (BGICC) Cairo-Egypt.	2018	2.0
3. 11th Breast and Gynecologic Immunotherapy Cancer Conference (BGICC)	2019	2.0

-Poster presentation in an international conference: (5)

18th ESGO meeting, Liverpool, UK	2013	1.0
19th ESGO meeting, Nice, France	2015	1.0
ESGO state-of-art meeting, Antalya, Turkey	2016	1.0
20th ESGO meeting, Vienna, Austria	2017	1.0
21th ESGO meeting, Athens, Greece (2 abstracts)	2019	1.0

-A speaker in a national conference:

1. The Annual conference of The Egyptian Society of Gynecologic Oncology (EGCS), Cairo	2014	1.0
2. The first Gynecologic Oncology Forum" of Mansoura University-Egypt. Mansoura and Sharm -Elsheikh, February	2014	1.0

3. The 8th annual conference of "The Annual conferences of Department of Obstetrics and Gynecology, Sohag University. Hurghada	2014.	1.0
4. The 8th annual conference of Department of Obstetrics and Gynecology, Suez Canal University	2015	1.0
5. The 9th annual conference of Department of Obstetrics and Gynecology, Sohag University	2016	1.0
6. The first Gynecologic Oncology meeting, Zagazig University	2017	1.0
7. The annual conference of The Egyptian Society of Gynecologic Oncology (EGCS)	2017	1.0

Attended meetings.

Weekly tumor board meetings, department of Clinical Oncology	2014-2019	3.0
First, 2nd, and 3rd GO meetings, Mansoura Oncology center	2016-2018	1.5

Teaching activities:

1. Undergraduate teaching, Faculty of Medicine, Mansoura University (Lectures-Clinical training-Assessment)	2012-2019	3.0
2. Course coordinator of Gynecologic Oncology. Mansoura-Manchester Program of Medical Education, Mansoura University	2015-2019	2.0
3. Postgraduate teaching (Gynecologic Oncology) to Master and Doctorate degree students. Mansoura Faculty of Medicine	201- 2019	2.0
4. Postgraduate teaching (Pelvic Anatomy) to Master degree students. Mansoura Faculty of Nursing	2015- 2019	1.0

Journal Editorial board membership.

World Journal of Clinical Case Conference (www.wjgnet.com).	2015-2018	1.0
Advances in Modern Oncology Research (www.advmodoncolres.com).	2015-2018	1.0

Reviewer:

1.The Lancet (1 peer review)	2019	1.0
2. International Journal of Cancer.(2 peer reviews)	2019	1.0
3. Journal of Pharmacology Research International. (1 peer review)	2018	0.5
4. Saudi Medical Journal. (1 peer review)	2016	0.5

5. Middle East fertility Society Journal (2 peer reviews)	2017	1.0
6. BMJ Open (1 peer review)	2019	0.5

Membership of organizations and societies.

A board member of Institutional Review Board (**IRB**) of medical research ethics, Mansoura University.	2016 -2019
International Society of Study of Trophoblastic Diseases (ISSTD); Officer for Egypt and North Africa	2019
Egyptian Gynecologic Cancer Society (EGCS).	2010-2019
European society of Gynecologic Oncology (ESGO).	2009-2019
International Gynecologic Cancer Society (IGCS).	2009-2017
Egyptian Society of Obstetrics and Gynecology.	2006-2019

Supervision of Master and Doctorate degree thesis:

Master degree of Obstetrics and Gynecology (5)	2007-2019	2.5
Master degree of Radiology (1)	2017	0.5
Master degree of Nursing (2)	2013-2018	1.0
Doctorate degree of Obstetrics and Gynecology (2)	2014-2019	2.0
Doctorate degree of Pathology (1)	2010-2014	0.5

Publications. **(from 2012 to 2019)**

A- In peer-reviewed international journals (Number: 20)

B- In peer-reviewed national journals (Number: 6).

Others

Initiation of Gynecologic Oncology Unit-Mansoura University	2012

12.4 List of publications.

1. **Reda A Ali**, Elsaid A, M M Elzayat. Multilocular intra-uterine cystic mass, atypical presentation of endometrial stromal sarcoma. A case report. Archives of Gynecology and Obstetrics,Vol. 277, number 2, Feb.2008:185-187.
2. **Hemida RA**. A proposal to improve treatment of gynecologic cancer treatment in the developing countries. Comprehensive review. Clinical ovarian cancer. Vol 2, No. 1 May, 2009: 38-43.
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12.5 Curriculum Vitae (C.V)

The author of this thesis was borne on September, 14, 1970 in Mansoura, Egypt. After finishing high school in 1988, he studied Medicine at Mansoura University, Egypt. He was graduated in November 1994 with "excellent with honor degree". In 1997, he was appointed as a senior house officer in the department of Obstetrics and Gynecology, Mansoura University Hospital. In 1999, he received his Master degree (MSc) in Obstetrics and Gynecology and started working as an assistant lecturer of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University. In 2006, he received his Doctorate degree (MD) of Obstetrics and Gynecology and was appointed as lecturer of Obstetrics and Gynecology. In 2008, he got a post-doctorate training grant at the Gynecologic Oncology unit, Erasmus MC, Rotterdam, the Netherlands. In January, 2012, he was appointed as associate professor of Obstetrics and Gynecology. In 2012, he initiated the Gynecologic Oncology unit in the department of Obstetrics and Gynecology at Mansoura.

In September, 2015, he initiated Mansoura Trophoblastic clinic as the first trophoblast-specialized clinic in Egypt. In March, 2017, he became a professor in Obstetrics and Gynecology in Mansoura University. In February, 2018, he was nominated as Northern African representative of International Society of Study of Trophoblastic Disease (ISSTD). His publications in the last years have been in the field of gestational trophoblastic disease.

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