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Research Paper

Circulating Cell Free DNA in the Diagnosis of Trophoblastic Tumors

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ABSTRACT

Gestational trophoblastic neoplasia (GTN) represents a group of diseases characterized by production of human chorionic gonadotropin (hCG). Since non-gestational tumors may occasionally secrete hCG, histopathological diagnosis is important for appropriate clinical management. However, a histopathological diagnosis is not always available. We therefore investigated the feasibility of extracting cell free DNA (cfDNA) from the plasma of women with GTN for use as a "liquid biopsy" in patients without histopathological diagnosis. cfDNA was prepared from the plasma of 20 women with a diagnosis of GTN and five with hCG-secreting tumors of unknown origin. Genotyping of cfDNA from the patient, genomic DNA from her and her partner and DNA from the tumor tissue identified circulating tumor DNA (ctDNA) (from 9% to 53% of total cfDNA) in 12 of 20 patients with GTN. In one case without a tissue diagnosis, ctDNA enabled a diagnosis of GTN originating in a non-molar conception and in another a diagnosis of non-gestational tumor, based on the high degree of allelic instability and loss of heterozygosity in the ctDNA. In summary ctDNA can be detected in the plasma of women with GTN and can facilitate the diagnosis of both gestational and non-gestational trophoblastic tumors in cases without histopathological diagnosis.

1. Introduction

Gestational trophoblastic neoplasia (GTN) is a spectrum of pregnancy related malignancies including invasive molar disease, choriocarcinoma and the much rarer placental site trophoblastic tumors (PSTT) and epithelioid trophoblastic tumors (ETT) (Hui et al., 2014). Prior to the development of cytotoxic chemotherapy these malignant conditions were invariably fatal. However, overall cure rates now exceed 98% due to the development of improved chemotherapeutic regimens and follow-up protocols (Seckl et al., 2010).

A hallmark of GTN is the production of human chorionic gonadotropin (hCG). Serum hCG levels aid rapid diagnosis and accurate disease monitoring (Seckl et al., 2013). However, hCG secretion alone is not always diagnostic of GTN as some non-gestational malignancies also secrete hCG (Iles et al., 2010). Women with GTN fall into two groups; (i) those who following evacuation of a molar pregnancy are treated with a clinical diagnosis of GTN based on rising serum hCG levels and (ii) those who present with an hCG-secreting tumor. For the second group histopathological examination of tissue is important for determining the correct diagnosis and when the diagnosis remains unclear molecular genotyping can play an important role (Fisher et al., 2007).

Most histological specimens of trophoblastic neoplasia are obtained via sampling of disease from the uterus. However GTN may be highly vascular and biopsy of tissue in the uterus or elsewhere may be deemed unsafe due to the risk of hemorrhage. Therefore some patients with metastatic disease, a raised serum hCG and characteristic history, such as a recent pregnancy, may be treated as a GTN without a histological diagnosis. This is because it is prudent to treat a highly curable disease, rather than risk morbidity and mortality via delay to achieve a histological diagnosis (Seckl et al., 2013). Patients who have GTN may therefore be left with uncertainty regarding their prognosis while patients with non-gestational tumors may be treated with inappropriate aggressive chemotherapy. For these women development of a blood based diagnostic test would be beneficial.

Circulating cell free DNA (cfDNA) has been investigated in patients with solid tumors and circulating tumor DNA (ctDNA) is reported to be detectable in a wide range of malignancies (Bettegowda et al., 2014). In solid tumors there is evidence that ctDNA is an effective biomarker at predicting relapse following surgery (Diehl et al., 2008) and progression during chemotherapy and targeted therapy (Diaz et al., 2012). In prenatal screening for aneuploidy, fetal cfDNA obtained from maternal plasma, has proven to be highly accurate with a detection rate for trisomy 21 of up to 100% (Norton et al., 2015). Since GTN is both a malignancy and pregnancy related we would predict cfDNA from trophoblastic cells to be present in the plasma of patients with these tumors. Due to the unique genetics of GTN i.e. the presence of

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non-maternal DNA in the tumor, the DNA signature of these tumors may be easily detectable. cfDNA may therefore provide unique genetic information about a patients' disease hereto unavailable.

This report describes the feasibility of extracting cfDNA from the plasma of women with GTN, detection of ctDNA within these samples and utility of cfDNA to act as a "liquid biopsy" to enable the correct diagnosis for patients with hCG-secreting tumors without a tissue diagnosis.

2. Methods

2.1. Patients

Twenty-five patients were enrolled in the study. All patients were in their first week of admission to the Trophoblastic Screening and Treatment Centre, Charing Cross Hospital (CXH) to receive chemotherapy for confirmed or suspected GTN. Patients were split into two groups according to presentation.

2.2. Group 1: Women with Confirmed GTN

This group included 20 patients, 18 of whom were previously registered with the Trophoblastic Screening and Treatment Centre, following a histological diagnosis of molar pregnancy and subsequently admitted to CXH for chemotherapy following a diagnosis of invasive molar disease. Two further patients with no previous diagnosis of molar pregnancy presented with metastatic gestational choriocarcinoma, confirmed on biopsy, and were included following admission for curative chemotherapy.

2.3. Group 2: Women with hCG-secreting Tumors of Unknown Origin

This group included five patients who were referred to CXH with raised hCG levels and tumors at one or more sites but no histological diagnosis. All five patients were treated with a presumptive diagnosis of GTN. Two of these patients are described below.

2.4. Case CFD-023

A 47 year old female presented with vaginal bleeding. Obstetric history included two normal male pregnancies 15 and 13 years previously and a termination of pregnancy 11 years ago. Routine chest radiograph revealed cannon ball lung metastases. Her serum hCG at this point was 374,365 IU/L. Two days following admission the patient was transferred to CXH. CT staging revealed a pelvic mass and multiple pulmonary metastases. MRI head showed a 2 mm brain metastasis. No tissue was available for diagnosis and her FIGO score was 20. The patient was initially treated with low dose induction etoposide and cisplatin (EP) (Agarwal et al., 2014) weekly and once clinically stable was switched to EP, methotrexate and actinomycin-D for patients with central nervous system disease (EP-EMA(CNS)) plus intrathecal methotrexate (MTX) as she had ultra-high risk disease (Seckl et al., 2013).

2.5. Case CFD-008

A 33 year old female presented with abdominal pain and a positive pregnancy test. Her obstetric history was a normal pregnancy four years previously and a miscarriage three years ago. Her medical history was of a T3N1M0 gastric adenocarcinoma successfully treated 18 months previously with neoadjuvant epirubicin, cisplatin, and capecitabine chemotherapy. She was initially managed locally as a suspected ectopic pregnancy but her serum hCG climbed to >200,000 IU/L. A computed tomography scan showed widespread liver metastases and she was therefore transferred to CXH. Further imaging revealed no other abnormalities. No tissue was available for confirmation of the clinical diagnosis of GTN and her FIGO score was 21. Initial treatment was with EP-EMA(CNS) plus intrathecal MTX.

2.6. Preparation of Plasma

Plasma was separated from whole blood by centrifugation at 1900 × *g* for 10 min at 4 °C. The plasma layer was separated and a further centrifugation step at 16,000 × *g* for 10 min was included. Plasma was stored at −80 °C until analysis.

2.7. cfDNA Preparation

cfDNA was prepared from 3 mL plasma according to manufacturer's instructions using a QIAamp circulating nucleic acid kit (Qiagen, UK).

Table 1
Non-maternal cfDNA in women with gestational trophoblastic tumors.

Case	Serum hCG (IU/L)	Days since administration of chemotherapy	Total cfDNA from 3 mL plasma (ng)	Estimated % of cfDNA that is ctDNA based on genotyping	Estimated ctDNA (ng)	Diagnosis based on ctDNA	Genotype of antecedent molar pregnancy
Post-mole trophoblastic tumors							
CFD-001	10,097	1	9.8	0	–		
CFD-002	12,946	6	17.4	0	–		
CFD-003	37,923	6	14.4	0	–		
CFD-004	35,095	3	6.0	18	1.08	GTN; post-CHM	Androgenetic, monospermic
CFD-005	1497	3	3.2	0	–		
CFD-007	448,650	6	8.6	42	3.61	GTN; post-CHM	Androgenetic, monospermic
CFD-009	14,884	2	24.2	0	–		
CFD-010	16,326	3	17.1	10	1.71	GTN; post-CHM	Not available
CFD-011	24,622	3	4.4	16	0.70	GTN; post-CHM	Androgenetic, monospermic
CFD-012	8308	2	5.9	0	–		
CFD-013	133,018	5	22.1	42	9.28	GTN; post-CHM	Not available
CFD-015	20,237	6	8.5	0	–		
CFD-016	30,227	2	16.2	12	1.94	GTN; post-CHM	Androgenetic, dispermic
CFD-018	238,703	7	4.6	13	0.60	GTN; post-CHM	Androgenetic, monospermic
CFD-019	53,046	0	5.4	0	–		
CFD-022	47,472	1	4.3	26	1.11	GTN; post-CHM	Androgenetic, monospermic
CFD-024	150,101	5	14.6	19	2.78	GTN; post-CHM	Androgenetic, monospermic
CFD-025	169,442	5	10.8	11	1.19	GTN; post-CHM	Androgenetic, dispermic
Post-term choriocarcinoma							
CFD-027	66,861	5	9.3	9	0.84	GTN	NA
CFD-031	700,855	1	23.7	53	12.60	GTN; post-male	NA

hCG, human chorionic gonadotropin; cfDNA, cell free DNA; ctDNA, circulating tumor DNA; CHM, complete hydatidiform mole; GTN, gestational trophoblastic neoplasia; NA, not applicable.