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Mola Invasiva – Special Form of GTD

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Abstract. Invasive hydatidiform mole is a relative rare form of gestational trophoblastic disease (GTD) Most of hydatidiform moles remit after evacuation but some of them have the tendency to invade the myometrium In some rare cases the tropoblastic tissue can be found in other tissues like lungs, vulva, vagina or broad ligament The aim of the study was to demonstrate some of clinical, immunohistochemical and DNA analysis findings of a patient with a previous diagnosis of a complete hydatidiform mole

Key words: GTD — Invasive mole — DNA analysis — Immunohistochemistry

Introduction

Gestational trophoblastic disease is a heterogenous group of various lesions including hydatidiform moles and true neoplasms like choriocarcinoma and placental site trophoblastic tumor (PSTT) The main characteristics of an invasive hydatidiform mole is the penetration of hydropic degenerated villi and trophoblastic structures deep into the myometrium or invasion into the uterine vasculature The majority of moles that becomes invasive are of the complete type In immunohistochemical staining with antibodies against HCG and cytokeratins, there is an intensive possitive reaction in trophoblastic cells (Mazur and Kurman 1994, Danihel et al 1994 a,b) A partial mole can also be invasive (Gaber et al 1986) In some rare cases molar tissue is transported through the bloodstream to extrauterine sites like lungs These "metastatic foci" are ussualy detected several weeks after the evacuation of a mole from the uterus but they may occur concurrently with a mole (Hsu et al 1962, Thiele and de Alvarez 1962, Paradinas 1997)

Materials and Methods

A 47 year old woman was curretaged because of metrorhagia and increased levels of beta HCG The result of histopathological examination and DNA analysis was – complete hydatidiform mole Because of USG finding of myomatous uterus, the age of the

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patient and increased beta HCG levels in blood (91, 876 mIU/ml) hysterectomy was performed a month later The patient was consultated in the Center of Trophoblastic Disease (Bratislava, Slovakia) We found a repeated rise of HCG levels (98,676 mIU/ml) and on a CT scan of the lungs there was a metastasis within the lower left pulmonary lobe (9 mm in diameter)

Histopathological examination formalin – fixed and paraffin embedded tissue was stained by conventional methods Immunohistochemical staining was performed using the primary antibodies to human chorionic gonadotropin -HCG (DAKO, Glostrup, Denmark) and cytokeratin (Monoclonal mouse anti cytokeratin AE1/AE3,Boehringer Mannheim, Germany)

DNA analysis Samples were obtained from uterus after hysterectomy Uterus was transported to laboratory under sterile condition – in physiological solution, with antibiotics – Penicillme G (Biotika, Slovakia) and Streptomycine (Antibiotic Co , Bulgaria), each 100,000 IU/1000 ml Blood samples from cubital vein, each 10 ml, from patient and her partner were taken into tubes with 0 5 ml EDTA as anticoagulant

1 Isolation of DNA DNA was isolated from chorionic villi of invasive molle by modified phenol-chlorophorm extraction (Miller et al 1988) High-molecular DNA was obtained from peripheral lymphocytes of the patient and her partner, by standard phenol-chlorophorm extraction (Inoko et al 1986)

2 PCR amplification of three variable number of tandem repeats (VNTR) regions in DNA

a) ApoB – hypervariable region is on a short arm of chromosome 2, on the 3′ end of gene for apolipoprotein B (2p24 – p23)
   Sequence of primers ApoB were
   ApoB1 5′ CCT TCT CAC TTG GCA AAT AC 3′ (20 bp)
   ApoB2 5′ ATG GAA ACG GAG AAA TTA TG 3′ (20 bp)
b) MCT 118 – hypervariable region is on a short arm of chromosome 1 (D1S80)
   Sequence of primers MCT 118 were
   MCT118A 5′ GAA ACT GGC CTC CAA ACA CTG CCC GCC G 3′ (28 bp)
   MCT118B 5′ GTC TTG TTG GAG ATG CAC GTG CCC CTT GC 3′ (29 bp)
c) COL 2A – hypervariable region is on a long arm of chromosome 12 (12q13 1)
   Sequence of primers COL 2A were
   COL 2AU 5′ CCA GGT TAA GGT TGA CAG CT 3′ (20 bp)
   COL 2AD 5′ GTC ATG AAC TAG CTC TGG TG 3′ (20 bp)

Components of the reaction mixture were placed into sterile microtube Total volume was 50 μl or 100 μl, respectively Microtubes were placed using preprogrammed algorythms into the thermal cycler – Programmable Thermal Controller (MJ Research INC, USA)

ApoB

Temperature	Time	Number of cycles
96 °C	2 min	
94 °C	1 min	
58 °C	6 min	30 cycles
72 °C	10 min	
4 °C	20 min	

MCT 118

Temperature	Time	Number of cycles
96 °C	2 min	
94 °C	45 s	
64 °C	45 s	28 cycles
70 °C	2 min	
72 °C	10 min	
4 °C	20 min	

COL 2A

Temperature	Time	Number of cycles
96 °C	2 min	
94 °C	1 min	
66 °C	5 min	25 cycles
72 °C	10 min	
4 °C	20 min	

Products of PCR (20 μl per sample) were detected by gel electrophoresis in 1 5% agarose gel in TBE buffer solution (89 mmol/l Tris, 89 mmol/l boric acid, 2 mmol/l EDTA, pH 8) at electric tension of 4–6 V/cm in 30 minutes DNA was visualisated by incorporated ethidium bromide (added directly into the gel in concentration of 0 5 μl ml^{-1}) that shined using UV transilluminator and documented by photography

Results

Histopathological findings besides the leiomyomas there was also an invasive hydatidiform mole with penetrating of the hydropic degenerated villi deep into the myometrium (Fig 1) There was an intensive positive immunohistochemical staining with antibodies against HCG and cytokeratins within the trophoblastic cells (Fig 2)

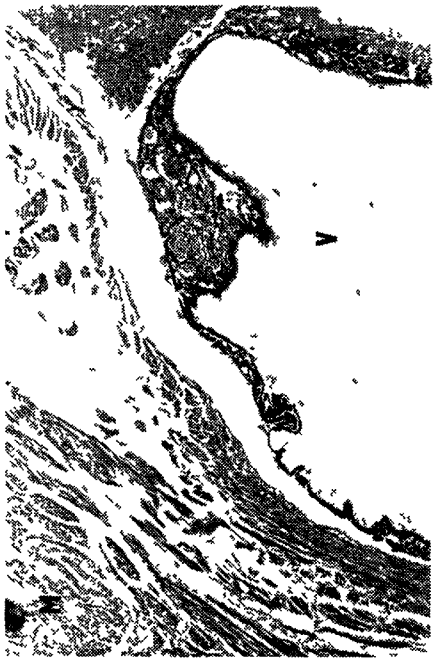

Figure 1 Invasive mole Hydropic villus deep within the myometrium M myometrium, chorionic vilus with proliferation of trophoblast on the surface HE 40×

Figure 2 Identification of HCG within the cytoplasm of syncytiotrophoblast Immunohistochemical staining with antibodies against HCG 100×

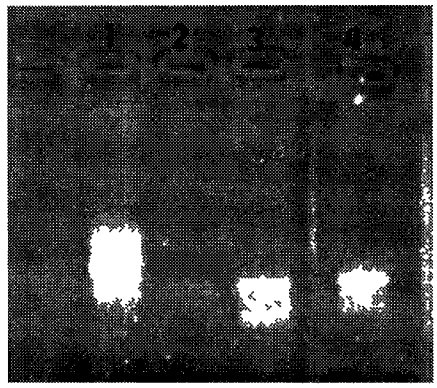

Figure 3. DNA analysis of invasive hydatidi form mole Patient (path 1), her partner (path 4), invasive mole from native material (path 3) and complete hydatidiform mole from fixed and parafine embedded material (path 2)

DNA analysis we obtained identical PCR products from invasive mole and from examinated father's (partner) DNA This proved, that the invasive mole originated from previous complete hydatidiform mole (Fig 3) We also tried to analyse DNA from fixed and parafine embedded material – we saw the token of a band identical to native material in path 3 Other VNTR systems used – MCT118 and Col2A – proved the same result

Discussion

Invasive mole is a possible sequela of hydatidiform moles Most of moles remit after evacuation but about 16% have the tendency to invade the wall of uterus or the chorionic villi together with trophoblastic cells can be deported to extrauterine sites (Mazur and Kurman 1994, Paradinas 1997) The villi with trophoblastic cells transported to extrauterine sites are not always an indication of neoplastic nature The trophoblastic structures enter the maternal bloodstream also in normal pregnancy (Covone et al 1984)

Before cytotoxic chemotherapy 4–15 % of patients with invasive mole died due to local complications like uterine perforation with intraperitoneal haemorrhage or metastases Nowadays patients with this type of GTD are treated very succesfully using cytotoxic chemotherapy

After 5 series of monochemotherapy (Methotrexate and Actinomycin D) the patient achieved a complete remission

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The Normal Female and the Male Breast Epithelium does not Express Prostate-Specific Antigen. Preliminary Immunohistochemical Observations of Autopsy Breast Tissues

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Abstract. In the normal female and male breast epithelial structures any prostate-specific antigen (PSA) immunohistochemical positivity was observed Variable PSA expression, which often borders the positivity, was observed in membranes of adipocytes of fat tissue and in the endothelium of small vessels in a female and a male breast Based on these initial observations, tissue of the normal breast, male or female, can not be considered to be the principal source of PSA

Key words: Prostate-specific antigen (PSA) — Immunohistochemistry — Normal female breast — Normal male breast

Introduction

Some investigators consider the female breast to be the principal source of PSA In female, not only the pathological breast tissue especially benign (hyperplastic) breast disease and cancer, but also the normal female breast tissue is assumed to be the principal source of

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