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CASE REPORT

Genetic prenatal RET testing and pregnancy management of multiple endocrine neoplasia Type II A (MEN2A): A case report

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ABSTRACT. Multiple endocrine neoplasia 2A (MEN 2A) is an inherited dominant syndrome characterised by medullary thyroid carcinoma, adrenal pheochromocytoma and hyperparathyroidism due to specific RET proto-oncogene mutations. Fertile MEN 2A women are at risk of complicated pregnancy because of unrecognised pheochromocytoma and transmission of RET mutation to the progeny. This condition may cause physiological distress in affected pregnant patients and their families. Here we describe the genetic prenatal testing, the pregnancy management and obstetric outcome in a MEN 2A patient with a right side adrenal hyperplasia and elevated calcitonin levels, a condition suspicious for possible recurrence of pheochromocytoma. We confirm that maternal or fetal complications are rare when MEN 2A diagnosis is made before pregnancy and an accurate monitoring is instituted. Furthermore, our results indicate that prenatal testing for RET mutations is highly recommended in making decisions and assuring parents on the lifelong risk of tumors. This will avoid the psychological distress that can further complicate the pregnancy of affected women.

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INTRODUCTION

Multiple endocrine neoplasia 2A (MEN 2A) is an inherited dominant cancer syndrome characterised by medullary thyroid carcinoma (MTC), adrenal pheochromocytoma and hyperparathyroidism (1). Related to this syndrome are MEN 2B, which includes MTC, pheochromocytoma, neurogangliomatosis and a marfanoid habitus, and familial medullary thyroid carcinoma (FMTC), in which the MTC is the sole clinical manifestation (1). Specific RET proto-oncogene mutations are associated with all three clinical entities: codon 634 is affected in most MEN 2A cases; codon 918 is almost uniquely altered in MEN 2B, whereas in FMTC more heterogeneous mutations have been described (2, 3). Pheochromocytoma is present in more than 50% of MEN 2A patients, but its frequency is probably underestimated, because it develops later in the follow-up and/or may be asymptomatic (4). Although classic features of pheochromocytoma are paroxysmal hypertensive crises or sustained hypertension with increased normetanephrine and/or metanephrine levels, most patients are in fact asymptomatic or have non-specific clinical manifestations and basal hormonal evaluation in the normal range (5). Fertile affected women are at high risk of complicated pregnancy because of the transfer of the mutated genetic trait to the progeny and the presence or development of pheochromocytoma that may render the pregnancy outcome or delivery difficult, resulting in high maternal and/or fetal mortal-

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ity (6, 7). Prenatal diagnosis and correct management of pheochromocytoma greatly improve survival and pregnancy outcome (6). Moreover, the RET test and the identification of specific mutations allow appropriate genetic counselling and prenatal diagnosis, avoiding psychological distress in affected pregnant patients and their families. Here we discuss the genetic prenatal testing and pregnancy management in a MEN 2A patient together with a reviewing of the literature.

CASE REPORT

A 31-yr-old primigravid woman affected by MEN 2A syndrome came to our Department for care at the 7th week of gestation. Detailed clinical description and genetic analysis have previously been reported (8). She had undergone total thyroidectomy (at 27 yr old) because of a MTC. Six months later she was hospitalised for hypertension, high plasma calcitonin levels and urinary catecholamine levels and underwent left adrenalectomy for ipsilateral pheochromocytoma. These features were in agreement with the diagnosis of MEN 2A with a peculiar clinical picture represented by MTC, calcitonin-producing pheochromocytoma and no involvement of the parathyroid glands (8). Molecular analysis of the RET gene disclosed an unusual condition with two RET mutations at codons 634 and 640, on two separate alleles. Endocrine evaluations performed before the pregnancy showed periodic upper-normal levels of catecholamines, increased calcitonin levels, normal calcium, phosphate and PTH (Table 1); ultrasound scan and computerized tomography revealed a hypertrophic right adrenal gland. In this peculiar case, elevated calcitonin (CT) levels and the right adrenal hyperplasia were suspicious for a pheochromocytoma recurrence in the right side. A glucagon test (1 mg ev) induced a near two-fold increase in plasma norepinephrine (115 to 226 pg/ml) and epinephrine (58 to 120 pg/ml), while heart rate and blood pressure were unchanged (72 and 110/80 before, 81 and 125/80 after). These data are consistent with no increased secretory activity from the hypertrophic right adrenal gland. Genetic counselling was provided and prenatal diagnosis for this new genetic variant of MEN 2A and for karyotype analysis was performed at the 11th week by chorionic villus sampling. The genetic analysis was carried out at the Department of Biochemistry and Medical Biotechnologies and Laboratory Medicine at the Medical School of the University of Naples Federico II, that is a reference centre for MEN 2 diagnosis for the entire region. All procedures were carried out after having obtained informed consent from the patient. The genetic test was performed on the DNA extracted from the chorionic villi and amplified by PCR using specific primers for RET exon 11. The PCR product was subsequently sequenced on both strands and the result excluded the presence of any RET mutation as those present in the mother's DNA. The result was confirmed by restriction enzyme digestion carried on the PCR amplified product. Karyotype analysis showed a 46XX value, without numerical and structural chromosome abnormalities. The patient maintained an euthyroid state, with 125 µg daily of oral levothyroxine and her blood pressure remained under 140/90 mmHg during pregnancy; urinary and plasma glycaemia, liver enzymes and electrolyte levels were also in the normal range throughout pregnancy. Urinary excretion of metanephrine was normal (Table 1). Plasma calcitonin levels remained elevated. The patient did not experience headache, tachycardia, irritability or edema. Ultrasound fetal examination showed normal growth. The patient underwent cesarean delivery at 39 weeks: the infant weighed 3590 g, with 1 and 5 min Apgar scores of 9 and 9. During surgery maternal blood pressure remained under 140/90 mmHg.

COMMENTS

Pheochromocytoma is a rare cause of secondary hypertension with an estimated incidence of 0.1% in the population affected by hypertension (9). Association of pheochromocytoma with pregnancy is

Table 1 - *Laboratory findings before, during pregnancy, and at delivery.*

Parameter	Before pregnancy	1st trim	2nd trim	3rd trim	Delivery	Normal range
Urinary epinephrine (µg/ml.24h)	10-14*	11	7.2	8	14.2	0-15
Urinary norepinephrine (µg/ml.24h)	81-95*	75	44.5	72	60	0-100
Urinary vanillylmandelic acid (mg/ml.24h)	4	5	3.2	3.6	3.8	2-7
Serum CT (pg/ml)	320	500	429		370	0-15
Serum PTH (pg/ml)	18		22			10-60
Serum TSH (µU/ml)	0.2			0.2		0.2-3.5

*: report of two observations; CT: calcitonin.

very rare, but its occurrence is responsible for high maternal and/or fetal mortality (7). It has recently been shown that prenatal diagnosis and correct management greatly improve survival and outcome drastically reducing maternal (1 vs 19%) and fetal (12 vs 25%) mortality (6). MEN 2A women are at risk of complicated pregnancy because of unrecognised pheochromocytoma. This tumor develops in more than 50% of MEN 2A patients and occurs bilaterally in nearly 50% of cases. However, the real incidence is underestimated because of the late occurrence of this neoplasia and because MEN 2A patients with a recognised MTC are not followed up for long time (4, 5). Pheochromocytoma is usually characterised by paroxysmal or sustained hypertension, headache, sweating, palpitation and skin pallor. Hypertensive crises, symptoms such as palpitations, nausea, sweating, headache, anxiety and depression, on the other hand, may be present in a normal pregnancy, due to a number of different causes. Basal hormonal evaluation may also be in the normal range (4). The correct diagnosis may then be difficult. Pheochromocytoma was, in fact, described as the "great mimic" for the numerous subjective manifestations and may be responsible for frequent psychiatric manifestations. Urinary metanefrine measurements and abdominal ultrasonography are the most useful tools for the diagnosis and localisation of pheochromocytoma also during pregnancy. However, basal urinary catecholamine excretion may be normal in many patients and tumor mass may be intra-adrenal. An altered epinephrine/norepinephrine ratio in 24-h collection and glucagon test have been suggested to improve the diagnostic sensitivity of catecholamine measurement (4, 9-11).

In the present case, adrenal hyperplasia and elevated CT levels could suggest a possible recurrence of a right side pheochromocytoma. In this patient we previously showed that the pheochromocytoma tumor expressed and secreted authentic calcitonin (8). Thus high calcitonin values after total thyroidectomy in absence of residual thyroid tissue, metastasised nodes and tissues, could indicate an unrecognised hyperplasia or recurrence of a medullary gland tumor. However, normal epinephrine/norepinephrine ratio and glucagon test performed before the pregnancy were both consistent with normal residual medullary function. The periodic assessment of urinary metanephrine and catecholamines and abdominal sonography scan together with normal clinical features (heart rate, blood pressure) reassured that pheochromocytoma did not recur during pregnancy and allowed us to plan a cesarean section without antiadrenergic pharmacological preparation. There is no general agreement on how to carry out the delivery (6, 12-14). We chose a cesarean section because the patient was primigravid and, as suggested by most authors, this method assures the control of delivery conditions. During pregnancy, therapy of pheochromocytoma was based on medical therapy with α-blockers to control maternal blood pressure. The α-blockers phenossibenzamine, prazosine or doxazosine are usually employed since none of these have any adverse reactions to the fetus or to the mother (14).

Reports of pregnancy outcome in MEN 2A patients are rare. Complications consisted of maternal or fetal death for hypertensive crisis due to sequelae of elevated catecholamine levels secreted from unrecognised pheochromocytoma. A maternal-fetal death for hypertensive crisis at 22 weeks (15) and a patient with hypertension, a right intracerebral hemorrhage at 35 weeks and residual neurologic deficits (16) have been described. When MEN 2A diagnosis precedes pregnancy and there is no incidence of pheochromocytoma, the pregnancy can proceed without maternal or fetal complications (17-19).

Another point to discuss is the importance of the genetic counselling and prenatal diagnosis in MEN 2A patients. Because it is an autosomally dominant disease, it manifests itself in a heterozygous condition, i.e. a single mutation in one RET allele is sufficient to develop the disease. Fifty percent of the progeny thus risks inheriting the mutation and developing the disease. RET mutations, moreover, have been associated with 3 risk levels from MTC and pheochromocytoma (20). RET codon 634 mutation, as the one described in the present case, is considered a level 2, with a high risk for MTC and justifies prophylactic total thyroidectomy before 5 yr of age. Moreover, pheochromocytoma has been associated with codon 634 mutation as early as 5 and 10 yr of age. Therefore, genetic prenatal testing for RET mutations is mandatory, even if risk and benefits should be discussed (20). A literature search showed only one report of prenatal diagnosis of RET proto-oncogene mutations in MEN 2A patients (21). Using chorionic villi as starting materials and the PCR and DNA sequencing test, we excluded that the fetus was bearing the same maternal mutation of RET protooncogene at 11 weeks.

In conclusion, pregnancy complicated by pheochromocytoma in MEN 2A is rarely described. The literature confirms that early diagnosis and collaboration between obstetricians, endocrinologists, anaesthesiologists and molecular biologists prevent the serious maternal and fetal complications associated with hypertensive crises. Prenatal testing for RET mutation and appropriate counselling are important in making decisions and assuring parents on the life-long risk of tumors, avoiding psychological distress that can further complicate pregnancy in affected women.

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