Adrenal Cushing's syndrome in pregnancy: Clinical and molecular characterisation of a case

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Abstract

Background: Cushing's syndrome is rare during pregnancy and more commonly due to adrenal pathology, in contrast to the non-pregnant population. Increased levels of cortisol-binding globulin and placental production of corticotropin-releasing hormone and adrenocorticotrophic hormone complicate the diagnostic strategies usually employed.

Case: A 32-year-old G1P0 at 15/40 gestation presented with severe peripheral oedema and excessive weight gain. Examination revealed pitting oedema to the abdominal wall, wide violaceous striae, moon facies and acne. Cortisol excess was confirmed with elevated 24 h urinary free cortisol, raised midnight salivary cortisol and lack of diurnal variation. Adrenocorticotrophic hormone ranged between 22 and 36 pg/ml (5–8 pmol/L). Fetal ultrasound confirmed a single live intrauterine gestation with an incidental finding of a maternal left adrenal mass. Magnetic resonance imaging confirmed an adrenal mass measuring 3.0 × 4.4 × 4.1 cm. She underwent a laparoscopic left adrenalectomy at 18 weeks' gestation without complication. Her postoperative cortisol level was undetectable. Hydrocortisone replacement was commenced with slow weaning as an outpatient. Histology was consistent with an adrenal adenoma. Immunohistochemistry revealed strong staining for the luteinising hormone/choriogonadotropin receptor, and expression of the luteinising hormone/choriogonadotropin receptor gene was in the range seen in normal ovary. DNA analysis revealed a mutation in *GNAS* encoding the G α subunit in the cyclic adenosine monophosphate pathway.

Conclusion: Cushing's syndrome may present in pregnancy as a result of β hCG acting on the luteinising hormone/choriogonadotropin receptor over-expression by the adenoma amplifying the aberrant cyclic adenosine monophosphate signaling implicated in the development of cortisol-secreting adenomas.

Keywords

Cushing's, pregnancy, adrenal adenoma, luteinising hormone/choriogonadotropin receptor, *GNAS*

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Case

Cushing's syndrome (CS) in pregnancy is uncommon; it is associated with high rates of maternal diabetes, fracture, pre-eclampsia, fetal morbidity and fetal mortality.¹ We report a case of Cushing's rapidly progressing in pregnancy and characterise the underlying molecular pathogenesis. This case illustrates the difficulties encountered in the workup of Cushing's in pregnancy and the complications that can ensue.

A 32-year-old Chinese woman, gravida 1 para 0, presented at 15 weeks' gestation with severe peripheral oedema and excessive weight gain of 30 kg over two months. She had been previously well, conceived naturally and was on no regular medications.

On examination, the most striking feature was pitting oedema to the top of her thighs, which hindered her mobility, with wide violaceous striae over her calves and lower abdomen (Figure 1(a)). She had a moon like facies with acne but no hirsutism.

Initial biochemistry revealed hypokalaemia (3.0 nmol/L; RR 3.5–5.0 mmol/L) with hypoalbuminaemia (2.5 g/dL; RR 3.5–4.5 g/dL). An abdominal ultrasound confirmed a single live intrauterine fetus with the incidental finding of a maternal left adrenal mass.

There was loss of diurnal variation of cortisol being 34.7 μ g/dL (958 nmol/L) at 8 a.m. and 34.1 μ g/dL (941 nmol/L) at 8 p.m. Adrenocorticotrophic hormone (ACTH) measurements ranged between 22 and 36 pg/ml (5–8 pmol/L). Her 24 h urinary free cortisol was markedly elevated at 2819 μ g/d (7782 nmol/d) with an elevated midnight salivary cortisol of 1.63 μ g/dL (50 nmol/L). An MRI scan confirmed an adrenal mass measuring 3.0 × 4.4 × 4.1 cm with no invasion into surrounding tissues or lymphadenopathy.

Insulin was commenced for her gestational diabetes. She underwent a laparoscopic left adrenalectomy at 18 weeks' gestation without complication. Histology showed an adrenal adenoma with no vascular or capsular invasion and no necrosis (Figure 1(b)). The mitotic count was low (less than 1 in 50 high powered field).

Her cortisol immediately postoperatively was undetectable at <0.4 μ g/dL (<11 nmol/L) and hydrocortisone replacement was commenced. Her peripheral oedema resolved rapidly.

A repeat obstetric ultrasound demonstrated a moderate sized cleft lip and palate. After extensive consultation, the patient opted for termination of pregnancy at 20 weeks' gestation. Subsequently she required metformin for ongoing diabetes and had developed severe depression requiring antidepressant medication. Nine months later, hydrocortisone has been weaned then ceased, her diabetes has resolved and her mood has improved. At one year follow-up, there is no biochemical or clinical evidence of recurrence.

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Figure 1. (a) Violaceous striae present on patient's calves and peripheral oedema. (b) H&E stain of adrenal adenoma, magnification 10 $\times$. (c) LHCGR immunohistochemistry, magnification 10 $\times$, inset negative control. (d) Expression of the LHCGR gene in the adenoma compared to normal ovary (n = 4).

Immunohistochemistry using an anti-luteinizing hormone/chorionic gonadotropin receptor (LHCGR) antibody revealed strongly positive staining of the adrenal adenoma (Figure 1(c)), consistent with the hypothesis that her Cushing's may have been driven by high β hCG levels in early pregnancy. Expression of LHCGR in the tumour was quantitated using qPCR demonstrating levels in the range seen in normal ovary (Figure 1(d)). Downstream of the LHCGR, activation of the cyclic adenosine monophosphate pathway (cAMP) has been implicated in multiple adrenal hyper-functioning conditions including cortisol producing adenomas. Thus tumour DNA was examined for the presence of the L205R mutation in cAMP dependent protein kinase A (PRKACA) and the activating codon 201 mutation in the G alpha subunit in the cAMP pathway (GNAS). While the sequence of this region of *PRKACA* was normal, *GNAS* was heterozygous for the codon 201 (CGT to TGT), Arg $\rightarrow$ Cys mutation.

Discussion

We report a case of escalating CS in pregnancy. Our patient had presenting features common to other cases such as gestational diabetes; however, what was striking was severe fluid retention and violaceous striae on her calves. In addition, the finding of a cleft lip and palate in association with CS has only been described on one previous occasion.² The high levels of cortisol in CS may have played a causative role, given epidemiological studies show an association between maternal corticosteroid use in early pregnancy and orofacial clefts.³

Increases in cortisol-binding globulin, plasma cortisol and placental corticotropin-releasing hormone (CRH) production confound the workup of CS in pregnancy. Urinary free cortisol increases through the second and third trimester, and suppression of plasma cortisol by 1 mg of dexamethasone is blunted in the standard overnight suppression

test.¹ Diurnal variation however is preserved, albeit with a higher night time nadir.

Contrary to the general population where pituitary pathology is the most frequent cause, adrenal adenomas account for more than half of cases in pregnancy.¹ To differentiate pituitary from adrenal causes, ACTH measurements and high-dose dexamethasone suppression tests (HDST) are traditionally employed. However, in one series, ACTH was non-suppressed in half of those with adrenal causes in pregnancy, postulated to be due to stimulation of pituitary corticotropes by placental CRH.¹ Furthermore, in the same series, three of seven patients with pituitary Cushing's did not suppress with the HDST.

Our patient's ACTH measurements were mid- normal non-pregnant range. Given the limited suppression of ACTH previously reported in pregnancy, and the presence of a large adrenal mass, we were confident that this was the source and did not perform further investigations for pituitary/ectopic sources of CS.

Our patient's adrenal mass would have been present for some time to reach 4 cm in diameter, yet she only developed clinical features of CS when she fell pregnant. Ectopic LHCGR expression in bilateral macronodular hyperplasia can present as CS in pregnancy. We thus postulated that over-expression of LHCGR on her adenoma could account for her explosive presentation in pregnancy. We confirmed this for both LHCGR protein and mRNA in the adrenal tissue. To our knowledge, only two other cases of adrenal tumours over-expressing LHCGR have been described as a cause of CS in pregnancy.^{4,5} Aberrant LHCGR has been described in association with the codon 201 mutation in *GNAS* in a female with CS who had a marked increase in cortisol secretion in response to exogenous hCG; however, this was not in the context of pregnancy.⁶ LHCGR is predominantly expressed in gonadal and reproductive tissue; however, LHCGR mRNA and

protein have been found in a wide variety of tissues including breast, prostate, skin, adrenal and neural retinal tissue.⁷

Aberrant activation of the cAMP pathway has been found in other hyperfunctioning adrenal conditions including bilateral macronodular hyperplasia and primary pigmented nodular adrenocortical disease. More recently, identification of the molecular basis of cortisol-producing adrenocortical adenomas has also implicated the cAMP pathway.^{8–11} In 35–60% of cases, somatic mutations were found in the catalytic subunit of the PRKACA.

In our patient, a mutation was found in *GNAS* encoding for the G α subunit in the cAMP pathway. In studies examining *PRKACA* mutations in adrenal adenomas, 5–17% of cases were found to have *GNAS* mutations.

In conclusion, CS can accelerate in pregnancy as a result of LHCGR over-expression amplifying the aberrant cAMP signaling downstream to this receptor implicated in the development of cortisol-secreting adenomas.

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Declaration of conflicting interests

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Ethical approval

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Guarantor

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Contributorship

All authors contributed equally.

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