Exacerbation of Cushing’s syndrome during pregnancy: stimulation of a cortisol-secreting adrenocortical adenoma by ACTH originating from the foeto-placental unit

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Summary

A 29-year-old G4A3 woman presented at 25 weeks of pregnancy with progressive signs of Cushing’s syndrome (CS), gestational diabetes requiring insulin and hypertension. A 3.4 × 3.3 cm right adrenal adenoma was identified during abdominal ultrasound imaging for nephrolithiasis. Investigation revealed elevated levels of plasma cortisol, 24 h urinary free cortisol (UFC) and late-night salivary cortisol (LNSC). Serum ACTH levels were not fully suppressed (4 and 5 pmol/L (N: 2–11)). One month post-partum, CS regressed, 24-h UFC had normalised while ACTH levels were now less than 2 pmol/L; however, dexamethasone failed to suppress cortisol levels. Tests performed in vivo 6 weeks post-partum to identify aberrant hormone receptors showed no cortisol stimulation by various tests (including 300 IU hLH i.v.) except after administration of 250 µg i.v. Cosyntropin 1–24. Right adrenalectomy demonstrated an adrenocortical adenoma and atrophy of adjacent cortex. Quantitative RT-PCR analysis of the adenoma revealed the presence of ACTH (MC2) receptor mRNA, while LHCG receptor mRNA was almost undetectable. This case reveals that CS exacerbation in the context of pregnancy can result from the placental-derived ACTH stimulation of MC2 receptors on the adrenocortical adenoma. Possible contribution of other placental-derived factors such as oestrogens, CRH or CRH-like peptides cannot be ruled out.

Learning points:

- Diagnosis of Cushing’s syndrome during pregnancy is complicated by several physiological alterations in hypothalamic–pituitary–adrenal axis regulation occurring in normal pregnancy.
- Cushing’s syndrome (CS) exacerbation during pregnancy can be associated with aberrant expression of LHCG receptor on primary adrenocortical tumour or hyperplasia in some cases, but not in this patient.
- Placental-derived ACTH, which is not subject to glucocorticoid negative feedback, stimulated cortisol secretion from this adrenal adenoma causing transient CS exacerbation during pregnancy.
- Following delivery and tumour removal, suppression of HPA axis can require several months to recover and requires glucocorticoid replacement therapy.
Background

Cushing's syndrome (CS) rarely occurs during pregnancy as increased levels of cortisol induces ovulatory dysfunction and relative infertility (1). Cushing’s disease (CD) is responsible for 70% of CS cases in non-pregnant patients (1); during pregnancy, primary adrenal adenoma, adrenocortical carcinoma, bilateral macronodular adrenal hyperplasia (BMAH) or primary pigmented nodular adrenal disease (PPNAD) represent 50–60% of CS cases (1, 2). It was suggested that androgen excess associated with CD suppresses more efficiently ovulation compared to primary adrenal lesions that produce less androgen secretion (1, 2).

CS is difficult to diagnose during pregnancy because of the overlap in the clinical features associated with CS and normal pregnancy. Also, placental-driven alterations in hypothalamic–pituitary–adrenal physiology during pregnancy complicate the diagnostic approach (2). It is primordial to diagnose CS during pregnancy because it is associated with significant materno-foetal complications and therapy decreases foetal loss and potentially maternal morbidity (1). Herein, we describe a patient with CS exacerbation during pregnancy that was secondary to a different mechanism than previously reported in cases of pregnancy-induced or exacerbated CS.

Case presentation

A 29-year-old G4A3 woman was found at 25 weeks of pregnancy to have a 3.4 × 3.3 cm right adrenal mass on abdominal ultrasound performed for acute nephrolithiasis. Starting 1 year before her pregnancy, she had only noticed modest weight gain, fatigue and lack of concentration. From 20 weeks of gestation, she noted purple stretch marks on her abdomen, facial rounding, supra-clavicular fat accumulation, dorsal fat pad, mild bilateral pedal oedema, mild proximal leg weakness associated with sciatica. She gained 32 pounds during her pregnancy. Her fasting blood glucose in the first trimester was 5.1 mmol/L, but she developed gestational diabetes during the second trimester and required 100U of insulin/day by 34-week gestation. She also developed gestational hypertension at 36-week gestation. At 25-week gestation, investigations revealed loss of diurnal plasma cortisol rhythm but only a slight increase in 24-h UFC. However, at 31 weeks of gestation, she had overt elevation of late-night salivary cortisol (LNSC) and 24-h UFC (Table 1). Corresponding plasma ACTH levels were not fully suppressed despite an 8.6-fold elevation of UFC (Table 1). Delivery was induced at 37-week gestation because of hypertension and intrauterine growth restriction. She delivered vaginally a 2.51 kg female baby. The baby required assisted positive pressure ventilation for 2 min immediately after birth and was found to have low morning serum cortisol values <80 nmol/L and hypocalcaemia-requiring therapy with prednisolone 0.1 mg i.v. twice daily and calcium supplements for several days. The child is now a healthy 3-year old.

One month post-partum, CS signs had regressed considerably in the mother with reduced appetite and normalisation of glucose levels without any therapy. Her 24-h UFC had normalised (107 nmol/day, Normal value (NV)<120) with corresponding suppressed ACTH of 1.4 pmol/L (NV: 2–11) (Table 1). Her morning plasma cortisol levels were not elevated, but did not suppress during a high-dose 4 mg i.v. dexamethasone suppression test (Fig. 1). Abdominal CT scan confirmed a 2.9 × 3.7 cm adrenal lesion with density of 20 Hounsfield Units (HU).

Investigation

The patient was evaluated with in vivo sequential tests to identify the presence of aberrant adrenal hormone

| Table 1 | Cortisol diurnal rhythm and values of urinary free cortisol (UFC), salivary cortisol and ACTH during pregnancy and 1-month post-partum. |
|------------------|------------------|------------------|------------------|
| Diurnal rhythm of cortisol (nmol/L) | 25-week gestation | 31-week gestation | 1-month post-partum |
| 08:00 h | 868 | 4338 (NR < 500) | 107 (NR < 120) |
| 23:00 h | 905 | 5 (NV: 2–11) | 1.4 (NV: 2–11) |
| 24 h UFC (nmol/day) | 787 (NR < 500)* | 15.7 (NV < 7) | 5.1 (NV < 7) |
| ACTH (pmol/L) | 4 (NV: 2–11) | 5 (NV: 2–11) | |
| Salivary cortisol (nmol/L) | | | |

*24-h urinary free cortisol were performed in different hospital laboratories during pregnancy and after delivery. NR, normal upper range of normal for non-pregnant woman are indicated in parenthesis. The 24 h UFC with the <500 nmol/day upper limit of normal were done with immunoassay and the 24 h UFC with the <120 nmol/day upper limit of normal was done by GCMS; NV, normal value.
receptors as previously described including an i.v. bolus of 300 IU recombinant human LH (hLH) and 2 months later an 100 mcg i.v. bolus of luteinising hormone-releasing hormone (LHRH) (3). *in vivo* screening tests to identify aberrant adrenal receptors (Fig. 2) showed a significant cortisol response (112% increase) following administration of 250 mcg of Cosyntropin i.v. and a partial response to vasopressin (Fig. 2B). There was no response to administration of recombinant human LH (Fig. 2C), LHRH, metoclopramide and TRH, to upright posture or mixed meal (Fig. 2A). To evaluate the expression of ACTH receptors (MC2R), LHCG receptors (LHCGR) and GnRH receptor (GnRHR), total RNA was extracted from the patient’s adenoma and compared to a pool of 11 normal adrenals (removed during radical nephrectomy) (NA pool). Quantitative RT-PCR analysis revealed detectable ACTH receptor (MC2R) mRNA levels, while LHCG receptor mRNA was almost undetectable (Fig. 3). GnRHR levels were similar to normal controls.

**Treatment**

Right adrenalectomy performed 7 months post-partum confirmed an adenoma (Weiss score: 0) with adjacent adrenal cortex atrophy.

**Outcome and follow-up**

She required glucocorticoid replacement post-operatively for 13 months. Normalisation of her HPA axis was documented by a 1 mcg Cosyntropin stimulation test (cortisol increased to 500 nmol/L from 344 nmol/L baseline) and baseline ACTH level of 4.4 pmol/L. Two years following adrenalectomy, she had a second pregnancy without diabetes, high blood pressure or CS. During her second pregnancy, LNSC measurements were less than 7 nmol/L, UFC levels were between 38 and 114 nmol/day (normal: <120 nmol/day) and plasma ACTH was between 4.0 and 4.5 pmol/L.
Discussion

In some cases, pregnancy induces or exacerbates hypercortisolism, and the aberrant expression of LH/hCG receptors in adrenal tumours or BMAH tissue was one identified mechanism (3). We report here a case of CS associated with an adrenal adenoma that was greatly exacerbated during pregnancy possibly by a different placental-related peptide such as placental ACTH, placental CRH or CRH-like factor since hypercortisolism regressed considerably post-partum.

CS diagnosis during pregnancy is difficult due to physiological changes in cortisol regulation which occur during normal pregnancy. Total plasma cortisol levels increase progressively due to oestrogen-driven enhanced synthesis of cortisol-binding globulin in the liver (2). In addition, plasma and UFC increase progressively during pregnancy due to the upregulation of the HPA axis (2). In normal pregnancy, ACTH increases by 2- to 3-fold in the second trimester despite elevated levels of free cortisol suggesting that there is another source of ACTH distinct from the pituitary that is not subject to negative feedback by cortisol (2). The most useful tools to diagnose CS in the context of pregnancy are 3-fold elevation of UFC above the upper limit of normal and loss of diurnal variation of serum cortisol (2). Jung et al. established trimester-specific normal values for 24-h UFC measures by LC–MS/MS in 20 healthy pregnant patients compared to non-pregnant patients (4). The mean 24-h UFC in normal pregnancy was increased by 2-fold and 3-fold in the second and third trimester, respectively (4). The 1 mg dexamethasone suppression test during pregnancy can be associated with false-positive results (2). Recently, Lopes et al. determined the best trimester-specific threshold for LNSC, based on sensitivity and specificity, to differentiate the normal pregnant women from non-pregnant CD: 7.0 nmol/L in the first trimester (sensitivity: 92% and specificity: 100%), 7.2 nmol/L in the second trimester (sensitivity: 84% and specificity: 98%) and 7.9 nmol/L in the last trimester (sensitivity: 80% and specificity: 93%) (5). Determining the aetiology of CS is also difficult during pregnancy since ACTH is only completely suppressed in half of the patients with primary adrenal aetiology of hypercortisolism (2). Our patient had a progressive increase in UFC value that reached more than 8-fold the upper limit of normal and a frankly elevated LNSC at 31 weeks of gestation. Her ACTH levels were not completely suppressed during pregnancy, despite a large excess of cortisol from her adrenal adenoma-causing severe CS.

In several cases of CS appearing or exacerbated during pregnancy, CS has improved or remitted after delivery and recurred in subsequent pregnancies (Table 2). In most cases, ACTH levels were suppressed (Table 2) suggesting that the stimulating factor bypassed the pituitary and stimulated the adrenal cortex directly. Several cases of pregnancy-induced CS associated with an in vivo stimulation of cortisol secretion by hCG were reported in the literature (Table 2). Most of these cases occurred with BMAH, but three cases of overt CS diagnosed during pregnancy were caused by an adrenal adenoma (6); in one, in vivo responses to hCG, LHRH, mixed meal, glucagon and vasopressin were demonstrated (6). The presence of LHCG receptor was demonstrated by immunohistochemistry (IHC) in the resected adrenal adenoma in two of these women (6). Recently, relapsing severe CS in two pregnancies was described in a woman with transient bilateral adrenal hyperplasia that remitted a few weeks after delivery (7); in vitro stimulation of cortisol production by LH and hCG was demonstrated in dispersed adrenal cells from this patient; the aberrant LHCGR remained expressed in BMAH cells after delivery which regressed in size between pregnancies (7). In other cases, in vitro studies by RT-PCR or IHC also showed an increased expression of LHCGR or GnRHR in BMAH (Table 2) (3).

Our patient’s evolution suggests that a factor from the foeto-placental unit aggravated hypercortisolism during pregnancy. We suspected the expression of an aberrant GPCR expression such as LHCGR or GnRHR in the adrenal adenoma. However, in vivo, recombinant hLH or LHRH did not induce a cortisol response in this patient. In addition, LHCG receptor mRNA was almost undetectable, and GnRHR receptor mRNA was similar to normal adrenal pool in RT-PCR of the patient’s adrenal adenoma.

The only significant response during in vivo testing was to exogenous ACTH 1–24. As ACTH was not fully suppressed during pregnancy compared to the post-partum period despite a large exacerbation of the cortisol production by the adrenal adenoma, this suggests that circulating ACTH was of placental origin (pACTH) which is not regulated by glucocorticoid-negative feedback and is bioactive in vitro (2, 8). In two other cases of adrenal CS that were exacerbated during pregnancy and associated with non-suppressed ACTH, expression of ACTH receptor or the response to Cosyntropin 1–24 were not evaluated (9, 10). Most adrenal adenomas associated with mild or overt CS can respond to exogenous ACTH 1–24 (3) as they maintain functional ACTH receptors despite increased
<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Diagnosis</th>
<th>ACTH</th>
<th>24 h UFC (times above upper limit of normal)</th>
<th>Plasma cortisol after 1 mg oral DST</th>
<th>Diurnal variation of cortisol</th>
<th>Post-partum evolution/recurrence</th>
<th>In vivo testing for aberrant hormone response</th>
<th>In vitro study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andreescu 2017 (6)</td>
<td>Adenoma</td>
<td>&lt;1.1 nmol/L</td>
<td>Elevated</td>
<td>360 nmol/L</td>
<td>Abnormal</td>
<td>Improvement and later underwent adrenalectomy</td>
<td>GnRH and hCG, meal, desmopressin, glucagon</td>
<td>ND</td>
</tr>
<tr>
<td>Plockinger 2017 (7)</td>
<td>BMAH</td>
<td>&lt;1.1 nmol/L</td>
<td>28</td>
<td>1194 nmol/L</td>
<td>ND</td>
<td>Remission</td>
<td>hCG, AVP</td>
<td>ND</td>
</tr>
<tr>
<td>Trinh 2016 (10)</td>
<td>Adenoma</td>
<td>5–8 pmol/L</td>
<td>Elevated</td>
<td>ND</td>
<td>Abnormal</td>
<td>Adrenalectomy during pregnancy</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Achong 2012 (16)</td>
<td>BMAH</td>
<td>&lt;1.1 nmol/L</td>
<td>7</td>
<td>ND</td>
<td>Abnormal</td>
<td>Remission and recurrence in multiple pregnancies</td>
<td>ND</td>
<td>hCG</td>
</tr>
<tr>
<td>Rask 2009 (17)</td>
<td>Adenoma</td>
<td>Suppressed</td>
<td>12</td>
<td>1738 nmol/L (1.5 mg DST)</td>
<td>ND</td>
<td>Adrenalectomy during pregnancy</td>
<td>ND</td>
<td>IHC for LHCGR</td>
</tr>
<tr>
<td>Chui 2009 (18)</td>
<td>BMAH</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Abnormal</td>
<td>Remission and recurrence in multiples pregnancies</td>
<td>hCG + LHCGR PCR elevated IHC LHCGR MC2R expression similar to NA</td>
<td></td>
</tr>
<tr>
<td>Hana 2001 (19)</td>
<td>BMAH</td>
<td>&lt;1.1 nmol/L</td>
<td>10</td>
<td>1200 nmol/L</td>
<td>Abnormal</td>
<td>Remission and recurrence in multiples pregnancies</td>
<td>hCG – ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kasperlik-Zaluska 2000 (20)</td>
<td>BMAH</td>
<td>Suppressed</td>
<td>ND</td>
<td>ND</td>
<td>Abnormal</td>
<td>Remission and recurrence in multiples pregnancies</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Wallace 1996 (21)</td>
<td>BMAH</td>
<td>Suppressed</td>
<td>Elevated</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>Remission but anormal diurnal variation persist</td>
<td>ND</td>
<td>Oestrogen containning oral contraceptive pill</td>
</tr>
<tr>
<td>Caticha 1993 (9)</td>
<td>PNAD</td>
<td>6.1–6.3 (N: 0–18)</td>
<td>31</td>
<td>1210 nmol/L</td>
<td>ND</td>
<td>Remission and recurrence in another pregnancy</td>
<td>Oestrogen</td>
<td></td>
</tr>
<tr>
<td>Close 1993 (22)</td>
<td>BMAH</td>
<td>Suppressed</td>
<td>ND</td>
<td>10</td>
<td>ND</td>
<td>Abnormal</td>
<td>Remission</td>
<td>ND</td>
</tr>
<tr>
<td>Reschini 1978 (23)</td>
<td>Adenoma</td>
<td>ND</td>
<td>ND</td>
<td>1170 nmol/L</td>
<td>Abnormal</td>
<td>Improvement</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Wieland 1971 (24)</td>
<td>Unknown</td>
<td>ND</td>
<td>ND</td>
<td>1170 nmol/L</td>
<td>Abnormal</td>
<td>Rapid improvement after abortion</td>
<td>hCG-oral contraceptve</td>
<td>ND</td>
</tr>
</tbody>
</table>

DC, dispersed cell stimulation in vitro; IHC, immunohistochemistry; ND, not done; PP, post-partum; PPNAD, primary pigmented adrenal nodular dysplasia.
cortisol production and in most cases undetectable ACTH levels (11). Whereas adjacent normal adrenal tissue and contralateral adrenal cortex downregulate ACTH receptors, ACTH receptors can be upregulated in the adenoma (11). This patient’s adenoma, resected during the post-partum period when overt CS had largely remitted, contained ACTH receptor mRNA, although levels were not as high as in the normal adrenal controls. Reincke et al. have demonstrated previously that some adrenocortical adenoma associated with mild CS express levels of ACTH-R mRNA lower than normal adrenal and that steroidogenic activity, measured by the expression of P450scc mRNA, correlates with the level of ACTH-R mRNA in the same tumour (12). Therefore, it is plausible to suggest that if the adenoma was resected during the period of overt CS, the expression of ACTH-R mRNA could have been higher. We suggest that pACTH or an ACTH-like substance stimulated the adenoma during pregnancy causing florid CS which regressed in the post-partum when ACTH levels became undetectable.

We cannot rule out the contribution of another factor derived from the foeto-placental unit such as CRH or a CRH-like substance that may have stimulated the cortisol-secreting adenoma directly (13). Placental CRH (pCRH) increases by a thousand-fold during pregnancy and was demonstrated to stimulate the secretion of peptides containing the ACTH sequence in primary culture of human placental cells (8). It potentially stimulates the HPA axis, although its biological activity is significantly limited by CRH-binding protein (2). It is impossible to rule out an effect from increasing oestrogen levels, which was previously shown to exacerbate CS in a woman with primary adrenal nodular dysplasia (PPNAD) during oral contraceptive use or pregnancy (9). IGF-2 secreted by the placenta could also have potentially contributed to the increased steroidogenesis through upregulation of steroidogenic key enzymes and ACTH receptor, but we did not determine IGF-2 or its receptor expression in this adenoma (14).

In conclusion, this case suggests a new regulatory mechanism underlying exacerbation of CS during pregnancy; while some cases of adrenal lesions with CS exacerbation during pregnancy were secondary to aberrant adrenal LHCGR expression, we propose that the normal MC2R present in cortisol-producing adrenomas can be stimulated by the physiological second trimester increase in pACTH. Due to the lack of specific pharmacological inhibitor of pACTH, we cannot exclude that other placental-derived hormones other than hCG could have also contributed to CS exacerbation during pregnancy.

Declaration of interest
Isabelle Bourdeau is a Senior Editor of Endocrinology, Diabetes and Metabolism Case Reports. Isabelle Bourdeau was not involved in the review or editorial process for this paper, on which she is listed as an author. The other authors have nothing to declare.

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Written consent was obtained from the patient for the publication of this report.

Author contribution statement
I B supervised the in vitro studies. A L is responsible for the in vivo and in vitro investigations and the medical care of the patient. All authors revised and approved the manuscript.

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