Hypercortisolism and pregnancy upregulate von Willebrand factor through different mechanisms: report on a pregnant patient with Cushing's syndrome

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Von Willebrand factor (VWF) is reportedly increased in pregnancy and Cushing's syndrome, inducing a hypercoagulable state. In Cushing's syndrome, VWF gene promoter polymorphisms modulate cortisol-dependent VWF upregulation, haplotype 1 (GCAG) and short GT-repeats $(GT)_S$ being the susceptible, and haplotype 2 (CTGA) and long GT-repeats $(GT)_L$ the protective pattern. We report on a Cushing's syndrome patient who became pregnant under hypercortisolism, in whom we monitored the evolution of her hypercoagulable state. During the active phase of Cushing's syndrome, the patient's VWF and factor VIII concentrations were normal, despite high urinary-free cortisol levels consistent with the presence of haplotype 2 and $(GT)_L$ alleles in the VWF gene promoter. VWF and factor VIII increased significantly and progressively after she became pregnant and peaked just before delivery, returning to normal 5 months later, while her hypercortisolism persisted. Our data indicate that two different mechanisms upregulate VWF under hypercortisolism and pregnancy, the latter being independent of the VWF promoter haplotypes sensitive to cortisol excess. ***Blood Coagul Fibrinolysis*** **21:476–479 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Pregnancy is rare in women with untreated Cushing's syndrome due to menstrual abnormalities occurring in approximately 80% of patients [1]. Severe maternal and fetal complications are reported in pregnant Cushing's syndrome patients, with higher rates of abortion, preeclampsia and fetal growth retardation [2]. Premature labor and fetal loss reportedly decrease in treated women, however, and some successful, uncomplicated pregnancies have also been described in untreated patients [3,4].

Cushing's syndrome is often associated with a hypercoagulable state due mainly to cortisol-induced upregulation of von Willebrand factor (VWF) [5,6], a multimeric glycoprotein mediating platelet adhesion at the site of vascular lesions and stabilizing circulating factor VIII [7]. In Cushing's syndrome, higher plasma VWF levels coincide with the presence of unusually large VWF multimers (ULVWMs), which are hemostatically the most active [8]; the resulting hypercoagulable state raises the risks of thromboembolic complications [9]. The effect of cortisol on VWF varies, however, because its action is modulated by particular VWF gene promoter polymorphisms: the −3268C>G, −2709T>C, −2661G>A, −2527G>A single-nucleotide polymorphisms, mostly segregating as haplotypes 1 (GCAG) and 2 (CTGA) [10,11], and the variable-length -2144$(GT)_n$ locus [12]. In Cushing's syndrome patients, haplotype 1 and short GT repeats (15–19 GT) are associated with higher VWF concentrations, whereas haplotype 2 and long GT repeats (≥20 GT) correlate with normal VWF levels [13,14].

Hypercoagulable conditions are described in normal pregnancy too, particularly near term and immediately postpartum [15]. VWF and factor VIII are known to increase progressively during pregnancy, with values higher than normal as of the 31st–35th weeks of gestation and reaching even 3–4-fold concentrations in the week before delivery, and returning to normal 1–2 months afterwards [16]. The molecular mechanism(s) behind pregnancy-induced VWF upregulation have yet to be clarified.

We report here on a young woman with Cushing's syndrome who became pregnant during the active phase of hypercortisolism. Her haemostatic profile was assessed in Cushing's syndrome and pregnancy, evaluating VWF and factor VIII variations and their relationship with the VWF promoter haplotypes.

Methods

The study was performed in accordance with the Helsinki Declaration, after its approval by our institutional review board.

Hemostatic and endocrine analysis

Plasma VWF antigen (VWF:Ag), collagen-binding VWF (VWF:CB) and factor VIII levels were measured

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according to previously published procedures [17]. VWF multimers were analyzed by autoradiography, as reported elsewhere [8]. Salivary and urinary-free cortisol were evaluated by RIA, using a commercial kit (DIA-Sorin Diagnostics, Saluggia, Italy).

Genetic analysis

Single-nucleotide polymorphisms −3268C>G, −2709T>C, −2661G>A, −2527G>A and the −2144$(GT)_n$ locus of the VWF gene promoter were analyzed from genomic DNA, using an ABI3100 Genetic Analyzer (Applied Biosystems), as explained elsewhere [12,13]. In the text, $(GT)_S$ stands for short GT-repeats (from 15 to 19 GT), and $(GT)_L$ for long GT-repeats (20 or more GT).

Results

A 29-year-old female suffering from Cushing's syndrome since she was 19 was studied. At diagnosis (1999), she had increased urinary-free cortisol (UFC) (Table 1) with a loss of cortisol circadian rhythm due to a pituitary microadenoma. Trans-sphenoidal surgery was successfully performed, but two years later she had recurrent Cushing's syndrome, cured with a second trans-sphenoidal neurosurgical procedure. After a transient remission she again presented clinical signs of hypercortisolism (Table 1) and she became pregnant.

Cushing's syndrome persisted throughout the pregnancy, as demonstrated by the lack of a circadian rhythm for salivary cortisol, the values of which were 9.26 ng/ml and 7.69 ng/ml at 11 p.m. in the 34th and 36th gestational week respectively (normal < 2 ng/ml).

The patient's hemostatic picture was assessed to monitor a potentially hypercoagulable state associated with Cushing's syndrome and revealed normal VWF:Ag, VWF:CB and factor VIII levels, (despite the high UFC levels) (Table 1). Her ABO blood group was type O.

Analysis of the VWF promoter polymorphisms, useful in predicting the risk of higher factor VIII and VWF levels developing in Cushing's syndrome, showed the $(GT)_{20}$/$(GT)_{21}$ genotype (i.e. homozygous L/L) in the $(GT)_n$ locus, which is reportedly not susceptible to the effects of cortisol on VWF [14]. As for the VWF promoter single-nucleotide polymorphisms, the patient carried the CTGA pattern (haplotype 2) reported to protect against any cortisol-induced increase in VWF, together with the GTGA pattern, an unusual haplotype generated by an uncommon segregation of the four polymorphisms [13].

Starting from the 34th gestational week, there was a progressive rise in VWF:Ag, VWF:CB and factor VIII concentrations, to more than double the pre-pregnancy levels by the 38th gestational week (Fig. 1a). A concomitant progressive increase in VWF multimers was observed, with no appearance of the unusually large VWF multimers which occur in Cushing's syndrome patients with high VWF levels (Fig. 1b). Labor was induced after the 38th gestational week. Two days postpartum, the patient's factor VIII, VWF:Ag and VWF:CB were still significantly higher than normal, but less so than before delivery (Fig. 1a). Follow-up tests 5 months later demonstrated a normalization of factor VIII, VWF:Ag and VWF:CB levels (Fig. 1a), and a normal multimer pattern (Fig. 1b), while the patient's hypercortisolism persisted (UFC = 761 nmol/24 h).

Discussion

We describe a young woman with longstanding Cushing's syndrome who became pregnant during the active phase of the disease. Her VWF and factor VIII levels, which had always remained normal during hypercortisolism, increased progressively during gestation, confirming that different pathways underlie the upregulation of VWF by cortisol excess and pregnancy.

VWF is an acute-phase molecule that increases temporarily in response to physiopathological conditions such as pregnancy, inflammation, diabetes, atherosclerosis, cancer and hormone therapy [18]. During pregnancy, VWF and factor VIII reportedly increase progressively, peaking at term: this is one facet of the major coagulative changes occurring in normal pregnancy, which shift the haemostatic balance towards a hypercoagulable state that protects women from life-threatening hemorrhage during delivery, but it also raises the risk of thromboembolic episodes [16]. Little is known about the mechanisms leading to pregnancy-induced alterations in the coagulative picture and the rising levels of pregnancy-related hormones do not seem to correlate with the increase in VWF levels [19].

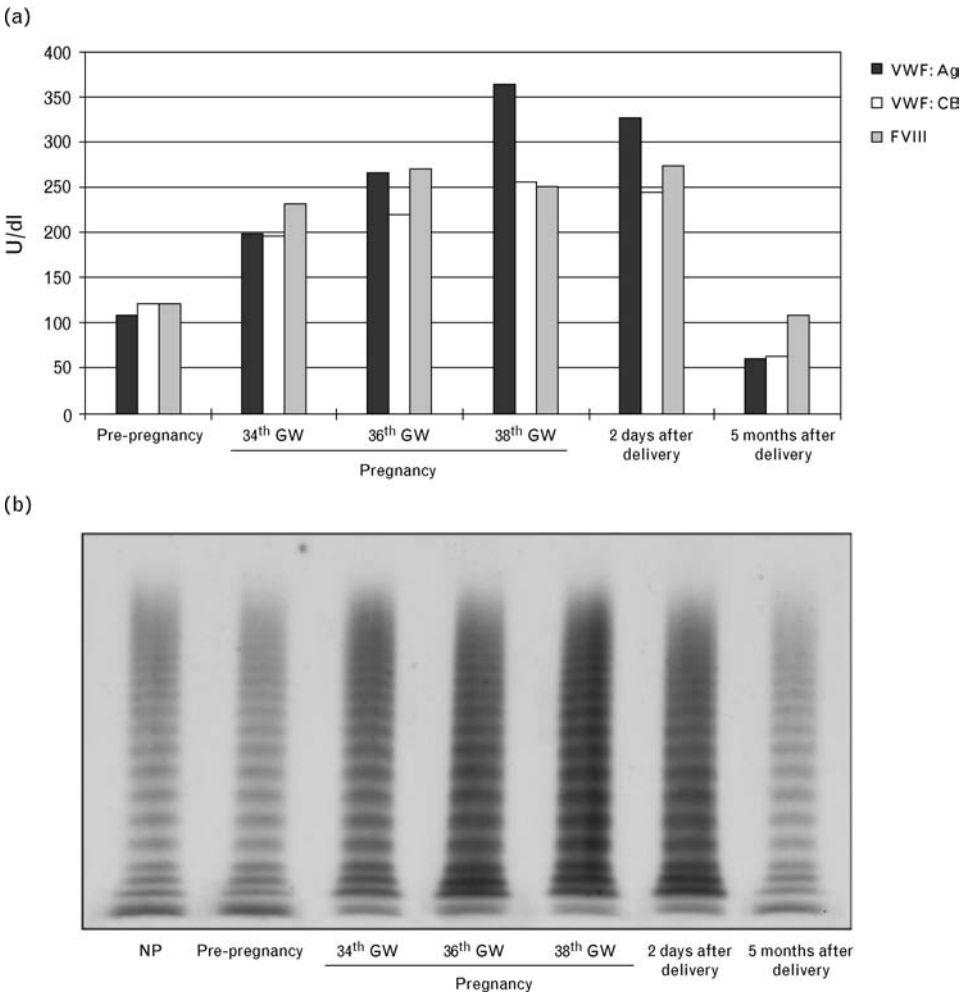
Cushing's syndrome is also associated with a VWF upregulation that correlates well with rising UFC concentrations even though the effect of cortisol on VWF is modulated by polymorphisms of the VWF gene promoter,

Table 1 Endocrine and haemostatic picture of the Cushing's syndrome patient before pregnancy

Year	Description	UFC (nmol/24 h)	VWF:Ag (U/dl)	VWF:CB (U/dl)	FVIII (U/dl)
1999	CS diagnosis	1462	95.4	83.7	128
2003	First recurrence	398	87.1	103.8	166
2004	Remission	52.4	117.3	101.8	90
2007	Second recurrence	1213.9	107.7	121.7	122
Normal range		37.8–270	60–160	65–150	60–160

CS, Cushing's syndrome; FVIII, factor VIII; UFC urinary-free cortisol; VWF-Ag, von Willebrand factor antigen; VWF-CB, collagen-binding von Willebrand factor.

Fig. 1



Time courses of factor VIII, von Willebrand factor antigen and collagen-binding von Willebrand factor (a) and von Willebrand factor multimer pattern (b) observed in the patient before, during and after pregnancy compared with normal pooled plasma (NP). Large VWF multimers are at the top, small multimers at the bottom. Note the full complement of multimers and the lack of unusually large von Willebrand factor multimers before and during pregnancy. VWF:Ag, von willebrand factor antigen; VWF:CB, collagen-binding von Willebrand factor; FVIII, factor VIII; GW, gestational week.

the combination haplotype 1-$(GT)_S$ coinciding with the susceptibility of VWF to cortisol, whereas haplotype 2-$(GT)_L$ is associated with a low risk of increasing VWF levels [13,14]. The patient was characterized by genotype $(GT)_L/(GT)_L$ on the VWF promoter, combined with one haplotype 2 (CTGA) and a second, uncommon haplotype (GTGA) that does not satisfy the linkage disequilibrium in the VWF gene promoter [12]. The patient's VWF and factor VIII levels were normal during all phases of hypercortisolism (as was to be expected, given her cortisol-insensitive VWF promoter polymorphisms), but they increased progressively during pregnancy (as seen in healthy women). This indirectly, but nonetheless clearly goes to show that the mechanisms upregulating VWF under cortisol excess are not the same as those raising VWF levels during pregnancy. Another noteworthy difference in VWF response to cortisol excess and pregnancy concerns multimer organization: no circulating unusually large VWF multimers were observed in our patient during pregnancy, despite her increased VWF levels, in contrast with findings in Cushing's syndrome patients whose elevated VWF levels are associated with ULVWM [8]. This discrepancy might reflect either different molecular mechanisms behind the increase in VWF, or a different origin of the VWF (i.e. the placental vessel wall) during pregnancy.

In conclusion, our data point to the existence of different molecular mechanisms underlying VWF upregulation in Cushing's syndrome and in pregnancy, the latter apparently being independent of the polymorphic arrangement of the VWF gene promoter sensitive to cortisol excess. These mechanisms will hopefully be better clarified in future, given the role of VWF in the onset

of prothrombotic states, and the numerous physiopathological conditions that induce VWF upregulation.

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