The relationship between stress and hair cortisol in healthy pregnant women

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Abstract

Purpose: Stress has been shown to cause a large range of adverse fetal effects. This pilot study is the first attempt to examine cortisol level in the hair of pregnant women and assess its potential as a biomarker of gestational stress.

Patients and Methods: Twenty-five healthy pregnant women, in whom hair cortisol levels and the Perceived Stress Scale (PSS) were measured and correlated.

Results: Maternal hair cortisol levels, ranging between 0.06 and 0.23 nmol/g of hair correlated positively and significantly with measures of perceived stress (ranging between 2-22); ($R_s=0.47$) ($P<.05$).

Conclusions: Our findings corroborate recent primate studies with induced stress, and suggest that hair cortisol is a potential biomarker of chronic stress in pregnancy. This new long term biological marker may have important implications in research and clinical practice.

Keywords: Cortisol, hair, stress, pregnancy

Stress can be defined as “a state of bodily or mental tension resulting from factors that tend to alter an existent equilibrium”.¹⁻² The normal human response to a stressor is modulated by several complementary systems, the principle components of which are the autonomic nervous system and the hypothalamic-pituitary-adrenal axis (HPAA).³

ACTH, upon release into the general circulation, binds to high-affinity membrane receptors on cells in the zona fasciculata of the adrenal cortex,⁵ rapidly inducing production and release of the glucocorticoid, cortisol.³ Subsequent to adrenocortical stimulation, ACTH acts on the hypothalamus to decrease the production of CRH, ultimately suppressing its own production in a negative-feedback.⁴

Cortisol exerts its effects by binding mainly to two types of cytoplasmic receptors: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs).⁵⁻⁷ Cortisol exerts negative feedback on HPAA activity (including its own production) at various sites, including the anterior pituitary, hypothalamus and hippocampus.⁸

Maternal stress reactivity has recently been the subject of considerable study. Several recent studies investigating gestational HPAA responses to stress have been conducted using laboratory stressors, administered in a controlled and standardized manner. The most commonly repeated outcome measures of HPAA activity in such tests include serum ACTH and serum cortisol.⁹⁻¹¹ Of note, given the involvement of the autonomic nervous system in the human stress response, many stress reactivity studies have also used

changes in blood pressure, heart rate and heart rhythmicity as outcome measures.

The risks of high levels of prenatal stress and anxiety have recently been the subject of vigorous study, focusing on the deleterious effects of maternal stress on the mother's mood, pregnancy outcome and fetal development.¹²⁻¹⁶ While many studies have been carried out in animals (*e.g.* rhesus macaques) in laboratory settings, a host of studies have also focused on human pregnancy outcomes and fetal development through the use of prospective, comparative studies. Data obtained from these investigations have clearly indicated that high prenatal stress is deleterious to the mother's affect, fetal development and pediatric long-term neurodevelopment.¹²⁻¹⁶

While saliva, blood or urine cortisol are appropriate measures of acute stress, there is a need for a biomarker of chronic stress. Recently, the use of hair analysis as a chronic state biological marker has been the subject of considerable study. Although the first reports of hair analysis for the detection of drug exposure date back to the mid-nineteenth century,¹⁷ the last 20 yr have seen hair analysis emerge as a valid tool for the detection of chronic xenobiotic exposure. As a result, hair analysis has become a valid screening tool for exposure to a number of drugs of abuse and is accepted as evidence in most judicial proceedings.¹⁸⁻²¹ A variety of drugs and endogenous substances have been accurately measured in hair, including: opiates, barbiturates, benzodiazepines, anabolic steroids, cocaine, nicotine and recently, cortisol and cortisone.²¹⁻²⁵ In clinical settings, hair drug levels have been used to assist in therapeutic drug monitoring.²⁴

A limited number of studies by the same principle authors have examined the incorporation and detection of cortisol in human hair.

The first of these investigations by Cirimele et al.²⁶ focused on the detection of 10 corticosteroids, one of which was cortisol, from the hair matrix. The investigators artificially infused hair matrix with a known quantity of each corticosteroid and then tested their ability to recover it using a high-performance liquid chromatography (HPLC) – ion mass spectrometry procedure (IMS). This study yielded promising results regarding this assay, indicating that it was able

to detect almost 70% of the cortisol infused into the matrix, with only 6% variation over repeated trials and a limit of detection of 0.04 ng/mg.²⁶ Of even more interest, this group also detected prednisone and beclamethasone in hair samples obtained as part of a forensic investigation, indicating that corticosteroids do deposit into hair and that they are detectable by currently available methods.

In the second of these studies, by Raul²⁷ 44 hair samples from 17 males and 27 females were assayed for the presence of cortisol and its 11 β -HSD 2 metabolite, cortisone, using a HPLC/mass spectrometry procedure. The limits of detection for cortisol and cortisone found in this study were 1 and 5 pg/mg respectively; extraction recoveries were 74% and 32% respectively. Cortisol concentrations found in the 44 hair samples ranged from 12 pg/mg to 163 pg/mg with a mean of 70 pg/mg. Also of note, these investigators found no influence of hair colour on cortisol or cortisone incorporation into the hair samples.²⁷

Very recently, the validity of measuring endogenous cortisol concentrations as a marker of stress has been documented in rhesus macaques. Animals exposed to chronic stress of relocation had a significant increase in mean hair cortisol (from 81.1 ± 7.5 pg/mL to 129.6 ± 15.5 pg/mg ($P < 0.001$)). As important, there was a correlation between saliva and hair cortisol levels ($P < 0.001$).²⁸

The objective of the present study was to investigate the correlation between stress experienced by healthy pregnant women and hair levels of cortisol corresponding to the same period in pregnancy.

Patients and Methods

This was a prospective, cross-sectional, cohort, pilot study.

Patients were recruited from callers to the Motherisk Program between February and July 2004. Most patients were located within 100 km of the primary study site (The Hospital for Sick Children, Toronto, Canada). Twenty-five healthy pregnant women between the ages of 18-45 yr. calling Motherisk requesting information regarding the safety of different non teratogenic medications during pregnancy were asked

to participate. The women were at the end of the first or beginning of the second trimester of pregnancy.

Chronic stress was measured using the Perceived Stress Scale (PSS). The PSS, created by Cohen et al²⁹ is the most widely used psychological instrument to quantify perceived stress. It is a short, 10 item questionnaire that does not require clinician administration and has been validated in obstetric populations. The results of the PSS are valid for between four and eight weeks before the date of administration.

The protocol for this study was approved by the Research Ethics Board at the Hospital for Sick Children in Toronto. Informed consent was obtained at the beginning of the follow-up call. Recruitment included medical history, history of medication use both during and one year before pregnancy, drug, alcohol and smoking history.

All patients were visited at their homes by a study investigator where the PSS was administered.

Hair cortisol analysis and cortisol quantification was performed using ELISA and extraction methods, also established previously by our laboratory.³⁰ Samples were prepared using methods previously by us.³⁰ Briefly, 1-1.5 cm of hair from the scalp end of each sample was cut and measured. At least 10 mg of hair were obtained from each hair sample for analysis. Hair was cut into small pieces, pulverized and added to 1mL of methanol. The hair-methanol mixture was then incubated for 45 minutes in a sonicator, removed and re-incubated at 50°C overnight, in a shaker. Samples were removed after incubation and 1mL of methanol was removed from each sample tube and placed in 1mL capacity Thermodyne® sample concentrator tubes. Methanol was then evaporated under a stream of N₂(g) until the samples were very dry. Following methanol removal, residues were re-suspended in 400 µL of phosphate buffered saline (PBS) at pH 7.2 and vortexed until well mixed.

Cortisol measurement was conducted using the Salivary Cortisol ELISA kit® (ALPCO Diagnostics, Windham, NH). The limit of detection of our ELISA assay was 1.14 ng/mL of cortisol at the 95% confidence limit.

Assessment of data distribution was done using the Shapiro-Wilks test for normality. Spearman's (Rank Order) correlations were employed to correlate values.

Results

Sufficient hair volume was available for analysis from all 25 subjects. Results from one hair sample (2%) were excluded from statistical analyses due to active/untreated polycystic ovarian syndrome (PCOS). A second patient was unavailable to complete PSS scores and so results from her hair sample were not included in correlational analyses, but are present as part of the hair cortisol descriptive statistics.

The women had average stress scale scores of 10.6 ± 5.81 (range 2–22). The PSS average scores in this group were indicative of a normal population with respect to their perceived stress levels.²⁹ Mean hair cortisol levels were 0.133 ± 0.048 nmol/g hair, range 0.064–0.234 nmol/g). Hair cortisol correlated with PSS, ($r_s=0.47$, $P<0.05$) (Figure 1).

FIGURE 1. Spearman's rank order correlation between PSS scores and Hair Cortisol levels in non-depressed patients ($n=23$). Spearman's correlational coefficient $r_s = 0.47$; 95% CI 0.07-0.74; $p<0.05$. A positive correlation between perceived chronic stress and hair cortisol levels, of virtually equally strength, was found in healthy pregnant patients ($r_s = 0.47$; 95% CI 0.07-0.74; $P<0.05$).

Discussion

This is the first study to correlate a validated measure of perceived stress with hair cortisol levels in humans in general, and in pregnancy in particular. These results corroborate the recent published data in macaques, where stress was induced and was shown to result in temporally-related increase in hair cortisol.²⁸

The PSS, developed by Cohen and Williamson in 1988²⁸ indicates that measures of perceived stress vary in accordance with sex, age and race. Cohen found that normal females, based on a sample of 1406 subjects, have a mean score of 13.7 ($\pm$ 6.6) on the PSS. Further, subjects between the ages of 18 and 44 yr score an average of 13.0 ($\pm$ 6.2) to 14.2 ($\pm$ 6.2) on the PSS. This scale has been validated for use in pregnant patients.²⁹

The consequences of high stress during pregnancy have been extensively studied over the past 20-30 years¹²⁻¹⁶ and it is evident that high levels of antenatal stress are associated with deleterious health outcomes for both the mother and the fetus.

Total, 24 hr urine, salivary and plasma free cortisol levels are well established in the literature as biological markers of HPA axis function. Measurement of cortisol and other indicators of HPA axis function are complicated by the sensitivity of HPA axis reactivity (the HPA axis becomes active in response to investigators or the study environment), as well as the difficulties inherent in HPA axis indicator sampling.

Pregnancy is a state of hypercortisolemia, due to hyperactivity of the HPA axis. Pregnancy is associated with up to 4-fold increases in total and free plasma cortisol;^{11,13} salivary cortisol is also elevated to 2-fold its non-pregnant values.^{11,13} Furthermore, decreases in cortisol binding globulin and increases in CRH during the last 4-6 weeks of pregnancy (due to drops in CRH-BP) may also contribute to much higher hair cortisol levels during pregnancy. Also of note, the magnitude of cortisol production changes between the pregnant and the non-pregnant HPA axis are not the same across all measures (*e.g.* total cortisol increases 4-fold, whereas salivary cortisol increases only 2-fold). Finally, pregnancy itself can be considered a highly stressful period, which likely contributes not only to

the magnitude of hypercortisolemia seen during pregnancy, but also to the large inter-individual variability seen in the measurements.

We detected a positive correlation between perceived chronic stress and hair cortisol levels was seen in the study group. These results appear to corroborate the hypothesis that cortisol responses to chronic stress.

Given the relationships between hair cortisol levels and perceived stress, our results support the potential of hair cortisol levels as clinical correlates of perceived stress. This novel test has recently received substantial experimental support. In male rhesus monkeys, hair cortisol levels, measured by ELISA increased by stress of relocation, returning to prestressor levels after adaptation to the new location.²⁸

Overall, this pilot study suggests that hair cortisol level has potential as biological marker for chronic stress during pregnancy. Further studies are required to elucidate the validity of hair cortisol levels as markers of perceived stress, the patient groups in which they are most useful as indices of disease and the various normal/disease reference ranges required for their utility.

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