CORTISOL/CORTISONE DOSAGES IN MATERNAL HAIR

DOSAGES CORTISOL/CORTISONE DANS LES CHEVEUX MATERNELS

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Table of contents

1 Assay objectives

The prevailing physiological mechanism considered in studies of prenatal stress and psychopathology is increased activity of the maternal stress-responsive hypothalamic-pituitary-adrenal (HPA) axis[1]. The HPA-axis responds to psychological or physiological stress with the production of corticotrophin-releasing hormone (CRH) from the hypothalamus, stimulating the hypophysis to produce adrenocorticotropic hormone (ACTH), resulting in higher production of cortisol from the adrenal glands. Cortisol can be metabolized into its inactive form cortisone by 11β- hydroxysteroid dehydrogenase (11β-HSD) type 2. The ratio of cortisone to cortisol is considered to be an indirect marker of 11β-HSD type 2 activity[2].

Hair cortisol (*HCC*) is increasingly used as a biological marker assumed to reflect the long-term endocrine consequences of chronic stress exposure as well as mental health disorders[3]. Compared to cortisol concentrations measured in saliva, urine, or serum, hair cortisol measures are less influenced by daily fluctuations due to circadian rhythms and fluctuations based on homeostatic regulation.
HCC provides a possibility to measure retrospectively cumulative cortisol levels of previous months to gain a more complete picture of the mean cortisol levels during the chosen period, via a single sample. The hypothesized way that hormone is incorporated into hair is largely through passive diffusion from systemic circulation during the formation of the hair shaft. With the generally accepted average hair growth rate of 1 cm/month, one or several segments of selected length can be analyzed for the mean levels of cortisol during the corresponding months[4].

The assessment of hair cortisone (*HCNC*) in parallel to HCC has been postulated to provide even more insight into the cumulative amount of active and inactive glucocorticoids in the body. For instance, higher levels of free cortisol could be due to a decreased conversion to cortisone rather than an increase in cortisol output. As such HCNC levels may provide a useful and robust alternative index of long-term HPA-axis activity[4]. Apart from the absolute levels of HCC and HCNC, the cortisone-to-cortisol ratio is also of interest, as it may reflect the 11β-HSD-activity[2,5].

The objective was to measure the levels of HCC and HCNC for each pregnancy trimester in maternal hair samples collected in the ELFE mother-child cohort. This set of biomarkers will be useful to study biological correlates to maternal prenatal stress and psychopathology, as well as linking them to children's subsequent development, stress reactivity and mental health.

2 Description of the assay method

Hair sample collection

The interviewing midwife collected maternal hair (about 60 hairs or 3 mm thick) from the occipital region, as close to the scalp as possible. The hair was stapled on labeled bristol board paper with the root-tip orientation noted. The whole set was placed in a labeled envelope and a follow-up sheet completed and attached to the sample. The hair was stored in the envelope at room temperature. 844 maternal hair samples were sent for analysis to Dresden Lab Services GmbH (http://www.dresden-labservice.de/) directed by Prof. Dr. Clemens Kirschbaum.

Corticosteroid measurements

To detect the different corticosteroids, high performance liquid chromatography tandem mass spectrometry (LC–MS/MS) was used. While in general LC-MS/MS methods are more difficult compared to immunoassays, they show high sensitivity, reproducibility, and little cross reactivity[6].

The extraction procedure and sub-sequent analysis of cortisol and cortisone has extensively been described elsewhere[6]. Briefly, hair samples were finely cut with surgical scissors in 3 cm hair segments, assuming to reflect cumulative cortisol secretion over the period of the preceding 3 months. Depending on women's hair length, this resulted in 1, 2 or 3 segments, corresponding to each pregnancy trimester, with the segment closest to the scalp representing the third trimester. Samples were washed in 3 mL isopropanol for 3 minutes (two times), and steroid hormones were extracted

from 7.5 mg of whole, non-pulverized hair using 1.8 mL methanol for 18 hours at room temperature. 1.6 mL of the clear supernatant was transferred into a new 2 mL tube. The alcohol was evaporated at 50°C under a constant stream of nitrogen and reconstituted with 225µL double-distilled water and 20µL of a mixture cortisol-d4, cortisone-d7, testosterone-d5, DHEA-d4, and progesterone-d9 as internal standards. Of this reconstituted extract, 100µL were injected into a Shimadzu HPLC-tandem mass spectrometry system (Shimadzu, Canby, Oregon) coupled to an SCIEX API 5000 Turbo-ion-spray triple quadrupole tandem mass spectrometer (SCIEX, Foster City, California) with purification by online solid-phase extraction.

The HCC and HCNC were calculated from the analyzed concentration (nmol/L), dilutions, unit conversion, and weighed hair sample. Reported concentrations are in pg cortisol/cortisone per mg sample weight. The lowest and highest detected concentrations of HCC and HCNC in ELFE hair samples were 0,11 pg/mg and 332,22 pg/mg, and 0,17 pg/mg and 81,37 pg/mg respectively.

Quality control

Inter- and intra-day variation

Inter- and intra-day variation were determined by calculating precision and accuracy estimates for hair samples with three replicates each on three separate days. The inter- and intra-assay coefficients of variance (CV) were below 10% for both cortisol and cortisone, and within the acceptable range (CV <15%)[6,7]. For 25 samples there was not enough material to repeat the analysis.

Limit of quantification

The lower limit of quantification (LLOQ) is dependent on the mass of hair used. Weighted mass of the ELFE hair samples ranged from 0.6 to 7.6 mg of hair. When normalized to 10mg weighed hair, the LLOQ values correspond to 0.09 and 0.07 pg/mg of cortisol and cortisone, respectively.

3 Data treatment

In addition to the detected concentrations of HCC (CORTISOL_Tx) and HCNC (CORTISONE_Tx) and their ratio (CCRATIO_Tx), we provide variables related to the hair mass (HAIRMASS_Tx) and hair length of the used sample (HAIRLENGTH_Tx). Down to 4.0mg hair mass, measurements are absolutely reliable; below that threshold assay precision is reduced, with increased variance in the data. In case of hair sample length less than 3 centimeters, and assuming a hair growth of 1 cm/month, it should be taken into account that the analyzed length does not correspond to the pregnancy trimester in its entirety.

Finally, we have added an indicator of the quality of the measure (QUAL_CORTISOL_Tx ; QUAL_CORTISONE_Tx). In some samples the levels of one or both corticosteroids could not be determined (Nd). For these samples, values in the dataset have been replaced with 0. However, it is important to note that for these samples the concentration of HCC and HCNC are very low and therefore not detectable with the applied method. Imputing 0 does not necessarily reflect the real concentration even if it should tend towards 0. We recommend using appropriate statistical procedures to manage these data points[8].

Please note that due to the non-normal distributions for HCC, HCNC and CCRatio concentrations, before any inferential analyses a preliminary step should be performed transforming the raw data with appropriate statistical methods, such as a log-transformation.

On a methodological level, HCC and HCNC have a relative robustness against potential confounders. Several studies have found only weak associations with age, season, hair washing frequency and hair treatment. In pregnant women, BMI, gestational age and birth weight have been found associated with maternal hair cortisol[9]. These confounders are available within the ELFE dataset and should be addressable through statistical adjustment[3].

Beyond covariates, many studies have found reduced corticosteroid concentrations in more distal hair

segments. HCC declines by 29% from the first proximal 3 cm hair segment to the second most proximal 3cm hair segment. As a central implication, this strongly suggests against conducting any pure within-subject comparisons of HCC or HCNC from different hair segments. In such a scenario it cannot be determined to which extent between-segment differences are due to actual exposure effects or, alternatively, merely an artifact of the natural segment decline. These two potential sources of influence can only be distinguished when the patients' pattern of between-segment HCC or HCNC differences is compared against the respective pattern of an unaffected control or reference group[3].

4 Conditions of data use

If these variables are used, cite the following in the acknowledgements: « The LC-MS/MS analyses were executed at Dresden Lab Services GmbH under supervision of Prof. Dr. Clemens Kirschbaum. The resulting hair Cortisol and Cortisone biomarker variables were constructed by Judith van der Waerden as part of the ANR funded ELIPSES project (ANR-17-CE36-0002-01) ».

5 List of variables

Cortisol_T1

Hair cortisol levels during first pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes :0.00-117.08)*

Cortisone_T1

Hair cortisone levels during first pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes : 0.00-56.05)*

CCRatio_T1

Cortisone/cortisol ratio first pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes :0.05-6.62)*

Hairmass_T1

Mass hair sample first pregnancy trimester

I__I__I__I.I__I__I mg *(Bornes : 0.90-7.50)*

Hairlenght_T1

Length hair sample first pregnancy trimester

I__I__I__I.I__I__I cm *(Bornes : 1.00-3.00)*

QUAL_Cortisol_T1

Information related to the quality of T1 hair cortisol concentration measure

1 : The concentration of T1 cortisol measured is above the LLOQ and CV are below 20% within the range of concentrations assayed.

2 : The sample was assayed but T1 cortisol could not be detected (Nd).

QUAL_Cortisone_T1

Information related to the quality of T1 hair cortisone concentration measure

1 : The concentration of T1 cortisone measured is above the LLOQ and CV are below 20% within the range of concentrations assayed.

2 : The sample was assayed but T1 cortisone could not be detected (Nd).

Remarks_T1

Additional remarks noted by the analyzing laboratory concerning the sample

String variable

Cortisol_T2

Hair cortisol levels during second pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes :0.00 -332.22)*

Cortisone_T2

Hair cortisone levels during second pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes : 0.00-58.75)*

CCRatio_T2

Cortisone/cortisol ratio second pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes :0.03-21.06)*

Hairmass_T2

Mass hair sample second pregnancy trimester

I__I__I__I.I__I__I mg *(Bornes : 0.60-7.50)*

Hairlenght_T2

Length hair sample second pregnancy trimester

I__I__I__I.I__I__I cm *(Bornes : 1.00-3.00)*

QUAL_Cortisol_T2

Information related to the quality of T2 hair cortisol concentration measure

1 : The concentration of T2 cortisol measured is above the LLOQ and CV are below 20% within the range of concentrations assayed.

2 : The sample was assayed but T2 cortisol could not be detected (Nd).

QUAL_Cortisone_T2

Information related to the quality of T2 hair cortisone concentration measure

1 : The concentration of T2 cortisone measured is above the LLOQ and CV are below 20% within the range of concentrations assayed.

2 : The sample was assayed but T2 cortisone could not be detected (Nd).

Remarks_T2

Additional remarks noted by the analyzing laboratory concerning the sample

String variable

Cortisol_T3

Hair cortisol levels during third pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes : 0.00-61.20)*

Cortisone_T3

Hair cortisone levels during third pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes : 0.40-81.36)*

CCRatio_T3

Cortisone/cortisol ratio third pregnancy trimester

I__I__I__I.I__I__I pg/ mg *(Bornes : 0.10-19.43)*

Hairmass_T3

Mass hair sample third pregnancy trimester

I__I__I__I.I__I__I mg *(Bornes : 1.00-7.60)*

Hairlenght_T3

Length hair sample third pregnancy trimester

I__I__I__I.I__I__I cm *(Bornes : 1.00-3.00)*

QUAL_Cortisol_T3

Information related to the quality of T3 hair cortisol concentration measure

1 : The concentration of T3 cortisol measured is above the LLOQ and CV are below 20% within the range of concentrations assayed.

2 : The sample was assayed but T3 cortisol could not be detected (Nd).

QUAL_Cortisone_T3

Information related to the quality of T3 hair cortisone concentration measure

1 : The concentration of T3 cortisone measured is above the LLOQ and CV are below 20% within the range of concentrations assayed.

2 : The sample was assayed but T3 cortisone could not be detected (Nd).

Remarks_T3

Additional remarks noted by the analyzing laboratory concerning the sample

String variable

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7 Annexes

Retour de données :

- livraison de la table (au format SAS si possible) des données de dosage avec l'identifiant des échantillons Elfe (NIPré)