Parity-related variation in cortisol concentrations in hair during pregnancy and in the postpartum period: a prospective cohort study

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Abstract

Objective: To investigate hair cortisol concentrations (HCC) monthly in pregnant women and to explore the effect of parity. Design: Prospective cohort study from gestational week (GW) 26, at childbirth and postpartum. Setting: An antenatal care clinic in southeast Sweden. Sample: 390 pregnant women. Methods: Cortisol was measured using radioimmunoassay in methanol extracts of ground hair samples. Main outcome measures: Hair cortisol concentrations Results: Both primi- and multiparae exhibited an increase in HCC throughout pregnancy. Primiparae had significantly higher HCC in the latter part of the last trimester compared to multiparae (one month p=0.003 and two months p=0.038). The use of psychotropic medication in the first trimester correlated to HCC postpartum (p<0.001). HCC in GW 14-17 were associated with HCC in GWs 18-21 (primiparae and multiparae, p<0.001), GW 22-25 (primiparae p=0.036 and multiparae p=0.033), and two months postpartum (primiparae p=0.049). HCC in GW 18-21 was associated with GW 22-25 among both primiparae (p<0.001) and multiparae (p<0.001) as well as two months prior to childbirth among primiparae (<0.037). In general, all estimates of HCC in pregnancy and postpartum showed a significant association between HCC for a specific month and the HCC in the previous month (all p<0.001), except for the association of HCC among primiparae in GW 22-25 and three months prior to childbirth. Conclusions: Increased cortisol concentrations in hair were observed during pregnancy, which decreased three months prior to childbirth in multiparae. The results indicate a quicker suppression of the hypothalamic CRH production by placenta CRH in multiparous women.

Introduction

The interest in the function of the hypothalamic-pituitary-adrenal (HPA)-axis of pregnant women is growing, as evidenced by the evolving literature revealing the influence of maternal stress on the offspring´s physical and mental health outcomes [1-5].

However, the evidence regarding whether prenatal maternal cortisol levels are the sole or main mediating link is conflicting[6]. It also seems that foetal vulnerability to high maternal cortisol levels varies across the gestational phases[4, 6-8].

Research regarding the impact of cortisol in the mediation of maternal stress upon the foetus is complicated by the fact that the HPA-axis activity shows substantial diurnal variability [9] in addition to individual differences. Single cortisol measures in plasma or saliva may therefore not sufficiently reflect the overall long-term biological activity of cortisol. Analysis of hair cortisol concentrations (HCC) is used to overcome such limitations[10-12], where one cm of hair corresponds to about one month´s cortisol accumulation[13]. This

method has been validated against cortisol in saliva across the pregnancy and postpartum periods and has proved to be a reliable metric of HPA activity, enabling estimation of integrated cortisol release[14].

Reports on HCC throughout pregnancy have revealed varying results, probably due to the different analysis methods used[11, 14-18]. The research focus has also been wide-ranging and has shown several factors of importance for HCC, including season, obesity[11,19] and delivery mode [11]. In addition, some [18-19] researchers have noted a relation between psychosocial and lifetime stress exposure on HCC during pregnancy, while others [20] have concluded that neither psychological distress, nor chronic stress or psychiatric symptoms had any impact.

Higher cortisol levels have been reported in primiparae versus multiparae[21-22], probably related to more pregnancy distress [23]. However, Federenko et al. in 2006 could not detect any effect of parity on cortisol levels[22].

HCC covering single-month periods may give more precise and more coherent results when investigating relations to childbirth and neonatal outcomes, but to our knowledge, no reports of measurements conducted on a monthly basis are available regarding HCC during pregnancy and postpartum. We hypothesised that there may be variations in cortisol hair levels during pregnancy and postpartum, possibly influenced by parity. Hence, the purpose of this study is to add to the knowledge of normal HPA-axis functioning during pregnancy and postpartum by determining HCC levels on a monthly basis. A second aim was to explore further differences in HCC levels associated with parity during pregnancy.

Methods

Sample

The Swedish antenatal health care system is used by almost 100 % of pregnant women in the country. The antenatal and delivery care is free of charge. At the antenatal care clinics (ANC) healthy pregnant women are recommended to attend the regular antenatal programme with seven to nine visits to a midwife during pregnancy, and, if needed, extra appointments with an obstetrician and/or with the midwife. Women attending the antenatal clinic at Värnamo Hospital, a small hospital in the south of Sweden, were asked to participate. They were given written and oral information about the study by their midwife at the first visit to the antenatal clinic around gestational week 12. At the next visit (around gestational week 26) those interested in participating signed an informed consent. A total of 953 women were enrolled at the antenatal clinic during the study period. Of these, 740 women were approached and asked to participate in the study, 154 were excluded due to difficulties in understanding Swedish, and 186 declined participation. During the study period, nine women did not provide the study nurse with any hair samples and one woman changed her mind and asked to be removed from the study. Thus, 390 women were included in the study. Samples of hair were taken at around gestational week 26, at childbirth and finally at the postpartum check-up. The numbers of hair samples of sufficient quantity and quality to perform HCC analyses were: taken around gestational week 26, n=330; at childbirth, n=303; and at the postpartum control, n=311 (Figure S1). The total sample was divided into primiparous women and multiparous women in most analyses. Demographic information as well as data on mental health and use of medications are presented in Table 1.

Patient involvement statement

At the time of the study no patients or patient organisations were involved.

Hair cortisol measures

HCC) were expressed as pg/mg with a method developed in-house using a competitive radioimmunoassay on methanol extracts of pulverised hair. A hair sample approximately 3 mm thick and 3 cm long was cut close to the scalp from the posterior vertex area of the head. The hair samples were further cut into 1.25 cm lengths to reflect the cortisol accumulations for each month, based on an assumption of an average hair growth rate of 1 cm per month (13). The hair samples analysed in this study weighed between 5 mg and 6 mg. In the laboratory, each sample was put into a 2 mL QiaGenRB sample tube along with a 0.5 mm QiaGen

stainless steel bead and weighed on a Sartorius MC 210p microscale. The samples were put in specially made aluminium cylinders accommodating five 2 mL Eppendorf tubes, and frozen in liquid nitrogen for two minutes. This was followed by mincing in a Retch Tissue Lyser II at 23 Hz for two minutes to produce a fine hair powder. The cortisol was extracted by adding 1 mL of methanol (Chromasolv, Sigma-Aldrich) to each tube and placing the tubes in a metal holder on a plate with a 5-degree inclination on a horizontal shaker at room temperature, keeping the steel beads in constant gentle motion within the tubes for a minimum of 10 hours. Finally, the tubes were centrifuged for one minute at 13000 rpm at +4°C in a microcentrifuge, Thermo Scientific Heraus, Picotm & Frescotm 17/21, and 800 µL of the supernatant was moved to another plastic sample tube for lyophilisation in a SpeedVac Plus SC210A (Savant) using an Edwards XDS 5 vacuum pump for at least three hours. The samples were dissolved in radioimmunoassay buffer and analysed as described by Morelius et al. 2004 (24). A hair sample of 3–10 mg is needed to maintain a total inter-assay coefficient of variation below 8% for the combination of hair extraction and measurement of cortisol by the radioimmunoassay. The intra-assay coefficient of variation for the radioimmunoassay itself was 7% at 10 nmol/L. The antiserum cross-reacts 137% with 5α-dihydroxycortisol, 35.9% with 21-deoxycortisol, and 35.9% with prednisolone, but less than 1% with endogenous steroids (15).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 26, IBM Inc., Armonk, NY, USA). The original HCC values were both divided into quintiles and also transformed into logarithm values. The associations between sociodemographic variables and parity as well as use of medications were tested by Pearson's chi^2-test. The medians of the original values were used as measures of central tendency and variation, respectively. Due to the non-normal distribution of the original HCC values, Spearman's correlation coefficient was used when determining whether cortisol levels were correlated with possible confounding variables. Multivariate linear regression analyses were based on logarithm-transformed values and were made to assess possible associations among the nine HCC measurements for each participant. A p-value $p < 0.05$ (two-sided) was considered statistically significant.

Results

Demographic data

The majority of the women were married or cohabiting, had been born in Sweden, had secondary or higher education and were gainfully employed. Significant differences were seen concerning age and educational background when comparing primiparae with multiparae. First time mothers were younger and had lower education. Tobacco use decreased during pregnancy among primiparae women but remained at a low level among multiparae women, see Table 1.

Cortisol concentrations during pregnancy; impact of parity

The median HCC for the total group of pregnant women during pregnancy, at childbirth and postpartum are shown in Figure 1. Primiparae had higher levels of HCC than multiparae at two months (p=0.038) and one month (p=0.003) prior to childbirth, see Figure 1.

Associations between monthly cortisol levels during pregnancy

A multivariate linear analysis performed separately in the total (see Table S1), primiparae (see Table 2a), and multiparae (see Table 2b), groups revealed significant associations between HCC at gestational weeks (GW) 14-17 with HCC at GW 18-21 (all groups: p<0.001) and GW 22-25 (total: p<0.001, primiparae: p=<0.001) and a tendency of association with the HCC at two months postpartum in the primiparae group (p=0.049). HCC at GW 18-21 was significantly associated also with HCC at GW 22- 25 (all groups; p<0.001). HCC at three months prior to childbirth was associated with the HCC two months prior to childbirth - (all groups: p<0.001). HCC at two months prior to childbirth was associated with HCC at one month prior to childbirth (all groups: p<0.001). HCC at one month prior to childbirth was associated with HCC at one (multiparae: p=0.003) and two months postpartum (multiparae: p=0.043). HCC at one month postpartum also including childbirth was associated with HCC at two and three months postpartum (p<0.001 in all

groups). Spearman's correlation revealed no associations between HCC levels with the use of steroids and analgesics nor any other medications, while the use of psychotropic medication correlated to HCC levels in all postpartum measurements (r=0.28, p=0.008), (r=0.23, p=0.003) and (r=0.30, p=0.004).

Discussion

Main findings

Measures of cortisol in hair clearly demonstrate an increase in cortisol levels throughout pregnancy with a peak around childbirth. This is in line with prior reports based on salivary cortisol, and supports the validity of measurement of cortisol in hair samples since these levels mirror the activity of the HPA axis throughout pregnancy. In the third trimester the increase in hair cortisol was significantly more pronounced in women expecting their first child compared to multiparae that showed a decrease in hair cortisol three months prior to childbirth.

Strengths and limitations

The possible uncertainty when extracting and measuring cortisol in hair samples has been minimised in this study since the applied method has previously been tested in several studies and proven valid[15,19,25]. Measurements of cortisol in hair instead of in saliva, blood, and urine have advantages, e.g. the measurements are non-invasive and hair samples can be stored at room temperature. There is also evidence for a high level of intra-individual stability in hair cortisol concentrations[11].

Several confounding factors should be discussed when interpreting cortisol levels, especially since previous research has shown conflicting results. Age and sex and to a weaker degree use of oral contraceptives, hair washing frequency and hair treatment may influence hair cortisol levels[26]. However, in our previous studies (unpublished data) frequent washing with shampoo, and the use of hair spray, gel and wax did not affect HCC levels, in line with a prior report[27]. On the other hand, the use of chemicals in hair treatments including; bleaching, dying, straightening, or permanent waves may interfere with the cortisol concentrations[27-28], although results are conflicting[11, 29]. Our use of radioimmuno- assay minimises the risks of confounding caused by treated, e.g. coloured, hair.

To our knowledge the highest possible physiological HCC level is unknown, and no clinical standards or reference values have so far been presented. An international interlaboratory process is ongoing to establish benchmark reference values[30]. The biological samples of HCC that could be considered extreme values in this study were replicated and analysed on two independent occasions giving practically identical results. The cortisol values were logarithmically transformed in the statistical analyses to reduce the variation possibly caused by extreme values.

A strength of this study was that the hair was cut into pieces as part of the analytical process in the laboratory to ensure that all analysed hair samples were from the latest month. Another strength is that pulverised hair was used in our analyses. Recent research has concluded that pulverising hair prior to hormone extraction is crucial[31].

In addition, the study is of a well characterised population. The attrition was mainly explained by language difficulties and therefore, we assume that it has negligible impact on data.

Interpretation

The observed progressive increase in cortisol levels during pregnancy is consistent with previous evidence of increased maternal mean cortisol level from about gestational week 25 - 28 as assessed by monthly measurement of cortisol in saliva [32]. Our present data indicate a slightly earlier increase of cortisol concentrations during pregnancy compared to previous studies as we found increased cortisol levels as early as gestational weeks 18- 21 [16]. This echoes the benefit of using HCC which reflects the average cortisol concentrations over extended periods of time. The previously established transient increase in cortisol levels associated with labour and the subsequent decrease during the subsequent four days[33-34] was not evident in our results. This was expected, as each measurement covers one month which makes short-lived fluctuations difficult to

measure using hair extracts, and the hair sample taken during labour also included one week before until three weeks after childbirth. Hence, the elevation of cortisol associated with labour was probably outweighed by the downswing in cortisol in the first three postpartum weeks, in agreement with prior findings[33, 35]. In our study, the cortisol levels at the first three months postpartum remained slightly higher than in the second trimester, consistent with earlier reports of prolonged cortisol elevation for two to three postpartum months [36-38]. This is in accordance with the reported suppressed dexamethasone test up to six weeks postpartum and blunted ACTH response to CRH up to 12 weeks postpartum [39] suggesting a slow restoration of the HPA system functions. It seems possible that ACTH has a role in the observed slow normalisation of cortisol level postpartum.

The present finding of higher cortisol levels among primiparous women in line with prior reports may reflect these women's worries, anxiety and stress about the upcoming labour [21-22, 40-42]. It is notable that the tendency for slowly escalating cortisol levels observed between weeks 14 and 25 is broken thereafter in multiparae, and the cortisol level becomes lower in the third month before childbirth, and then increases again following the same pattern as for primiparae but remaining lower. The explanation for this could be a more rapid inhibition of the mothers´ hypothalamic secretion of CRH by the placenta CRH secondary to adaptive processes during the longstanding inhibition during previous pregnancies. The influence of parity on the cortisol level vanished directly after the childbirth and remained that way, consistent with previous observations on salivary cortisol levels[22].

The postpartum cortisol levels, were not related to those in the former part of the second trimester but rather to those from about GW 18 until childbirth, a period when the placenta CRH is becoming more dominant in the regulation of the rising cortisol level [32]. This suggests that the HPA-axis has not fully normalised in the first three postpartum months but is still under the influence of pregnancy-related HPA alterations.

Taken together, the results suggest that the HPA axis may respond differently to the placenta production of cortisol in multiparae. One explanation could be that the negative feedback system is more prepared and suppresses the cortisol production of the adrenal cortex quicker and more effectively secondary to the initiation of cortisol placenta production. Such a theory would not only explain the lower concentration of cortisol in multiparae but also the lack of association between cortisol levels in GW 14 -17 and the levels found in the periods when placenta production has started. Similarly, in primiparae, the concentrations are dominated for longer by the woman's own secretion, thereby explaining the closely related monthly cortisol levels of the second trimester and the lack of relation to the levels found in the third trimester.

Conclusion

Monthly measurements of HCC appear to closely mirror the activity of the HPA-axis during pregnancy. Increasing cortisol concentrations were found during pregnancy, with a decrease three months prior to childbirth in multiparae. The results suggest a quicker suppression of the hypothalamic CRH production by placenta CRH in multiparous women.

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Contribution to authorship

IM had the original idea for the study.

GS, ÅF, ET and AJ planned and performed the study and interpreted the results.

All authors contributed to the interpretation of the data and revision of the manuscript, and gave input at all stages of the study

Details of ethical approval: The study was approved by the Regional Ethical Review Board in Linköping nr 2011/499-31 (13-03-12).

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Table 1. Sociodemographic data and characteristics for primi- and multiparas

	Primipara		**Multipara**		**p-value**
	n=125	%	**n=232**	%	
Age-groups					<0.001
15-24 years	25	20.0	21	9.1	
25-34 years	94	75.2	167	72.0	
35-39 years	6	4.8	36	15.5	
40 or older	0	0.0	8	3.4	
Civil status					0.438
Married/co-habited	125	100	226	98.7	
Partner	0	0.0	2	0.9	
Single	0	0.0	1	0.4	
Ethnicity					0.191
Swedish	115	93.5	207	91.2	
European	8	6.5	14	6.2	
Non-European	0	0.0	6	2.6	
Education					0.055
Primary school	2	1.3	3	1.6	
Secondary school	44	35.5	110	47.6	
University college	43	34.4	51	22.1	
University	36	28.8	67	29.0	
Employment					

	Primipara		**Multipara**		**p-value**
Employee	85	68.0	146	62.7	0.314
Self-employed	6	4.8	12	5.2	0.885
Student	5	4.0	9	3.9	0.949
Unemployed	5	4.0	9	3.9	0.949
Long sick-leave	1	0.8	1	0.4	0.654
Tobacco use					
Before pregnancy	48	20.9	2	20.8	0.988
Until GW 25	12	5.2	1.6	1.6	0.094
Medication until GW 25					
Steroids	3	9.4	12	16.9	0.316
Psychotropic	4	12.5	12	16.9	0.568
Analgesic	3	9.4	14	19.7	0.191
Other	27	84.4	45	64.3	0.039

GW = gestational week

Other – folic acid, iron, proton-pump inhibitors, antihistamines, aspirin, thyroxine

Table 2a: Multivariate analysis of cortisol concentration (HCC) in relation to each of the nine measurements, for **primiparae**(log values)

	GW 14-17	GW 18-21	GW 22-25	3 months prior to childbirth	2 months prior to childbirth	1 month prior to childbirth	Childbirth and 1 month postpartum	2 months postpartum	3 mo
	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Sta coe cie Be (p- val
GW 14-17	-	0.810 (<0.001)	0.181 (0.036)	0.258 (0.166)	-0.060 (0.472)	-0.093 (0.362)	0.237 (0.116)	0.276 (0.049)	0.1 (0.
GW 18-21		-	0.705 (<0.001)	-0.072 (0.756)	0.085 (0.406)	0.103 (0.414)	0.263 (0.137)	-0.087 (0.595)	-0. (0.
GW 22-25			-	-0.052 (0.798)	-0.018 (0.841)	-0.025 (0.819)	-0.086 (0.578)	0.023 (0.870)	0.1 (0.
3 months prior to childbirth				-	0.905 (<0.001)	0.119 (0.378)	0.241 (0.160)	-0.256 (0.107)	-0. (0.
2 months prior to childbirth					-	0.744 (<0.001)	-0.075 (0.702)	0.201 (0.262)	0.2 (0.
1 month prior to childbirth						-	0.417 (0.003)	0.273 (0.043)	0.1 (0.

	GW 14-17	GW 18-21	GW 22-25	3 months prior to childbirth	2 months prior to childbirth	1 month prior to childbirth	Childbirth and 1 month postpartum	2 months postpartum	3 mo pos
Childbirth and 1 month postpartum							-	0.508 (<0.001)	0.5 (<
2 months postpartum								-	
3 months postpartum									-

GW = gestational week

Table 2b: Multivariate analysis of cortisol concentration (HCC) in relation to each of the nine measurements for **multiparae**(log values)

	GW 14-17	GW 18-21	GW 22-25	3 months prior to childbirth	2 months prior to childbirth	1 month prior to childbirth	birth and 1 month postpartum	2 months postpartum	3 mo pos
	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Standardised coeffi-cients Beta (p-value)	Sta coe cie Be (p- val
GW 14-17	-	0.561 (<0.001)	0.390 (<0.001)	0.205 (0.033)	-0.080 (0.232)	0.012 (0.822)	0.025 (0.809)	0.046 (0.388)	0.0 (0.
GW 18-21		-	0.211 (<0.001)	-0.105 (0.269)	0.136 (0.037)	-0.014 (0.793)	0.086 (0.448)	-0.101 (0.089)	-0. (0.
GW 22-25			-	0.152 (0.099)	0.036 (0.577)	-0.034 (0.520)	0.135 (0.169)	-0.009 (0.857)	-0. (0.
3 months prior to childbirth				-	0.740 (<0.001)	-0.037 (0.567)	0.138 (0.296)	0.037 (0.591)	-0. (0.
2 months prior to childbirth					-	0.875 (<0.001)	0.230 (0.198)	0.013 (0.886)	0.0 (0.
1 month prior to childbirth						-	0.024 (0.856)	0.014 (0.845)	0.0 (0.

	GW 14-17	GW 18-21	GW 22-25	3 months prior to childbirth	2 months prior to childbirth	1 month prior to childbirth	birth and 1 month postpartum	2 months postpartum	3 mo po
Childbirth and 1 month postpartum							-	0.875 (<0.001)	0.8 (<
2 months postpartum								-	
3 months postpartum									-

GW = gestational week

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HCC Figure.200616.docx available at https://authorea.com/users/335624/articles/461482-parity-related-variation-in-cortisol-concentrations-in-hair-during-pregnancy-and-in-the-postpartum-period-a-prospective-cohort-study