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Short Communication

Maternal Prenatal Stress and 4–6 Year Old Children's Salivary Cortisol Concentrations Pre- and Post-vaccination

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In this study influences of maternal prenatal stress on the cortisol reactions of children to a vaccination were determined. Prenatal stress at around 16 weeks of gestation was measured through questionnaires and a cortisol day curve. Cortisol reactions were determined preceding and following the vaccination. A total of 24 children (age between 3.11 and 5.9 years, mean age 4.9 years) and their mothers participated in this study. Multilevel analysis (hierarchical linear modelling) was used to analyze the data. Children of mothers who had higher concentrations of morning cortisol during pregnancy had higher concentrations of cortisol as compared to children of mothers who had lower concentrations of morning cortisol. Furthermore, more daily hassles and a higher level of fear of bearing a handicapped child during pregnancy were associated with higher concentrations of cortisol in the children.

Keywords: Children; Prenatal stress; Psychosocial stress; Salivary cortisol; Vaccination

INTRODUCTION

Maternal prenatal stress has been shown to induce overactivity and/or dysregulation of the HPA-system in animal offspring (Barbazanges *et al.*, 1996; Huizink *et al.*, 2004; Mulder *et al.*, 2002). Similar findings on the effects of prenatal stress on behavior and physical development of non-human primates have been obtained (Schneider *et al.*, 1999). In our prospective longitudinal study in humans, we found adverse effects of prenatal maternal stress on temperament and behavior of very young children (Huizink *et al.*, 2002). However, it is not yet clear whether maternal prenatal stress influences endocrinological stress reactions of human offspring.

The present paper presents the results of a follow-up study on the influence of prenatal maternal stress on children's cortisol reactions to vaccination stress. Children of mothers who had experienced more prenatal stress at the 16th week of pregnancy were expected to display higher cortisol reactions and/or overall higher concentrations of cortisol than those of less prenatally stressed mothers.

MATERIALS AND METHODS

Participants

Subjects were recruited from a population of pregnant women of the University Medical Center Utrecht (UMCU) in Utrecht, The Netherlands.

For this study a total of 24 mother-child pairs were available and willing to participate (19 girls and 5 boys). There was no selective attrition except for gender: the subjects were 19 girls and 5 boys. The children were between 3 years and 11 months old, and 5 years and 9 months old (mean age = 4 years and 9 months, SD = 0.66). The project was approved by the hospital Ethics Committee. Parents and children participated on a voluntary basis, with parents giving written informed consent.

Independent Variables during Pregnancy

Three different stress scores measured with questionnaires were used as predictors for the stress reaction of the children. First, daily hassles were measured by means of

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the Everyday Problem List (Alledaagse Problemen Lijst, Vingerhoets *et al.*, 1989). Second, pregnancy-related anxiety was measured by two scales: Fear of bearing a physically or mentally handicapped child (4 items) and fear of giving birth (3 items), which are part of the Pregnancy Related Anxiety Questionnaire-Revised, PRAQ-R (Van den Bergh, 1990; Huizink, 2000). Only these two scales were used because earlier results were found for these scales (Huizink *et al.*, 2004). Third, perceived stress was determined by the Dutch version of the Perceived Stress Scale (Cohen and Williamson, 1987).

Furthermore, maternal prenatal cortisol day curves were determined by collecting saliva every 2 h between 8 AM and 8 PM. Salivary cortisol is a valid and reliable reflection of the unbound hormone in blood (Kirschbaum and Hellhammer, 1989).

Dependent Variables of the Child

Confounders

To ensure that possible differences between prenatally stressed and non-stressed children were not due to the circadian rhythm of cortisol, food intake, use of medication and/or sleep patterns, mothers were asked to fill in a diary on the vaccination day. All diaries showed that the children had not eaten or slept for at least one hour prior to the saliva collection and that the children were in good health and had not used medication on the sampling days.

Cortisol Sampling

Children collected saliva at the following time points: upon entering the room where they would receive the vaccination (baseline sample) and 15, 20, 25 and 30 min after the vaccination (response samples). In order to obtain control values of cortisol in the form of a day curve, the parent(s) helped the child to collect saliva on 1 day in the weekend prior to the vaccination. The day curve consisted of 5 samples taken every 3 h between 8:00 AM and 8:00 PM.

Cortisol Determination

Oral stimulants, such as sweet powdered drink crystals, were not used in this study because data suggest that may compromise the validity of assay values (Schwartz *et al.*, 1998).

Mothers were instructed to store the saliva samples in a refrigerator and upon conclusion of the collection, mail them to our lab. Mailing does not affect the cortisol levels (Clements and Parker, 1998). Samples were stored then at -20°C until analyses. All samples from a single child were assayed in the same batch. The cortisol concentrations were measured without extraction, using an in house competitive radioimmunoassay with polyclonal anti-cortisol-antibody (K7348). $[1,2\text{-}^3\text{H(N)}]\text{-Hydrocortisone}$

(NET 185, NEN-DUPONT, Dreiech, Germany) was used as a tracer after chromatographic verification of its purity. The lower limit of detection was 0.5 nmol/l and inter-assay variation was 10.0, 6.4 and 6.0% at 5.1, 11.9 and 20.6 nmol/l, respectively ($n = 98$).

Data Analysis

Stress measures collected during the first period of pregnancy, at 15–17 weeks of gestation, were chosen as independent variables for the postnatal reaction to the vaccination because in this period the maternal prenatal stress levels were not significantly related to each other. Secondly, the amount of maternal prenatal missing data was the smallest as compared to the other two periods.

Missing Data

Overall 28% of the children's cortisol samples were missing due to children or parents who forgot to collect saliva, or to the amount of saliva collected being too small for cortisol detection.

Statistical Analysis

Children were placed in high and low prenatal stress groups by median split for each prenatal stress variable.

Multilevel Analysis calculations (Hierarchical Linear Modelling) were carried out with the aid of MLWiN (Goldstein *et al.*, 1998) in order to examine the influence of prenatal stress on the reaction of the children to the vaccination. The multilevel model was used because of the hierarchical structure of the data and because 28% of the samples of the children's data were missing, which made performance of ordinary repeated measure analyses inadequate and precluded using other methods such as area under the curve (AUC). The model consisted of two levels: the subjects were level 2 and the cortisol measures on the vaccination day were level 1. The prenatal stress variables, the time of sampling, sample number, and gender were introduced into the model one-by-one. Variables were kept in the model when their presence resulted in a significant ($p = 0.05$) reduction of the likelihood-ratio statistic.

RESULTS

Paired samples *t*-tests showed that the basal concentration of cortisol on the vaccination day was not significantly different from that during the weekend (weekend sample mean = 7.42 nmol/l, basal sample mean = 8.72 nmol/l, $t = -1.03$, $p = 0.32$, two-tailed). The final multilevel statistical model is shown in Table I.

The negative effect of the "sample number" means that samples taken earlier in the 5-sample sequence had higher values than samples taken later in the sequence. There was a small non-significant reaction, and overall the cortisol

TABLE I Estimates for the “best” multilevel statistical model of the 4-6 year old children’s salivary cortisol data, and the influence of maternal prenatal stress (pregnancy weeks 15-17: daily hassles, fear of bearing a handicapped child, early morning salivary cortisol concentration), sample number and time of sampling on the day of vaccination, and gender. Only variables with significant effect, $p < 0.05$, are shown

	Coefficient	SE
<i>Fixed effects</i>		
Intercept	-10.69	7.84
Sample number	-0.19	0.02
Time of sampling	-0.31	0.74
Daily hassles	4.54	3.20
Fear of bearing a handicapped child	1.22	3.16
Early morning cortisol of the mother	6.72	3.22
Gender	7.26	3.56
<i>Random effects</i>		
Level 2 (i.e. subjects) variance intercept	29.75	11.72
Level 1 (i.e. sample) variance intercept	5.62	1.21
Time of sampling variance	-0.55	0.12

concentrations decreased from the pre-vaccination concentration to the concentration taken 30 min post vaccination (Figure 1).

Furthermore, there was more variance in the child’s salivary cortisol concentration in the 15, 20 and 25 min samples after the vaccination than in the child’s basal cortisol concentration (see also Figure 1). Also, as was to be expected, vaccinations earlier in the day were related to higher cortisol concentrations than those that took place later. Higher maternal prenatal early morning cortisol was related to higher concentrations of children’s cortisol in reaction to the vaccination. Also, if the mother had reported more daily hassles during the first period of pregnancy, her child tended to have higher concentrations of cortisol on the vaccination day than children of

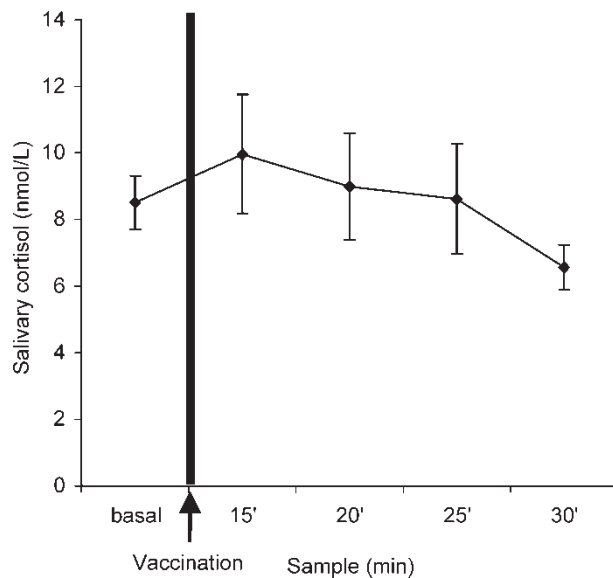


FIGURE 1 Children’s salivary cortisol concentrations (Mean, SEM) before and after a vaccination. Values are cortisol concentrations in nmol/l pre- and post-vaccination (Mean, SEM). Basal: $N = 18$ (8.51, 0.81); 15 min: $N = 16$ (9.97, 1.78); 20 min: $N = 18$ (8.98, 1.60); 25 min: $N = 18$ (8.62, 1.66); 30 min: $N = 16$ (6.56, 0.67). There was no significant salivary cortisol response to vaccination.

mothers who had reported less daily hassles during pregnancy. The same result was found for maternal fear of bearing a handicapped child. Higher levels of fear of the mother in pregnancy were related to higher concentrations of cortisol in the child on the day of the vaccination. Finally, there was a trend for girls in our study to have higher concentrations of cortisol than boys.

DISCUSSION

Several prenatal stress factors, measured at 15–17 weeks of gestation, were found to be related to children’s cortisol concentrations in saliva before and after a vaccination at ages 4–6 years. Children of mothers who had higher concentrations of salivary cortisol at 8 AM prenatally (at 15–17 weeks of gestation) had higher overall concentrations of cortisol as compared to children of mothers who had lower concentrations of morning cortisol. Furthermore, more maternal daily hassles and a greater fear of bearing a handicapped child were associated with higher overall concentrations of salivary cortisol in children on the vaccination day. These results support our hypothesis that children of mothers who had experienced more prenatal stress would display overall higher concentrations of cortisol than those of less stressed mothers.

The results must be interpreted with caution, given the limitations of our study. We did not use a genetic informative design and could therefore not investigate the influence of genetic factors on the results. Furthermore, we did not measure concurrent stress levels of the mother or her parenting skills, and were unable to examine whether these factors affected the child’s overall cortisol concentrations. The hypothesis that children of mothers who had experienced more prenatal stress displayed higher cortisol reactions to vaccination could not be accepted, as prenatal stress could not be linked to a significantly higher reactivity to the supposed stressor. Rather, the cortisol reaction to the vaccination proved to be insignificant and was followed by an overall decline in cortisol concentrations within the 30 min post-vaccination. An explanation for the absence of a significant reaction of cortisol to vaccination may be that the peak concentration of cortisol occurred later than 30 min post-stressor (Goldberg *et al.*, 2003). However, because our overall result was a decrease of the cortisol concentrations, a peak reaction after 30 min would appear highly unlikely in our data set. Moreover, as Gunnar and Donzella (2002) already stated, it seems to be difficult to stimulate an increase in salivary cortisol, on average, in response to mildly threatening situations in toddlers and preschoolers. It is possible that other, more potent, stressful situations in a child’s life could unveil differences in cortisol reactions between children of prenatally stressed and non-stressed mothers.

Finally, our results showed a trend for girls to have greater saliva cortisol concentrations than boys. Although

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this same gender difference was found in an earlier study (Watamura *et al.*, 2003), it should be noted that there were more girls than boys in our study.

The overall conclusion of this study is that even after 4 to 6 years, differences in levels of maternal experience of prenatal stress are positively related to children's basal salivary cortisol concentrations.

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