Morning Salivary Cortisol in Young Children: Reference Values and the Effects of Age, Sex, and Acute Bronchiolitis

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Objective To identify morning salivary cortisol reference values in infancy and at 2 years of age and to investigate the influence of age, sex and acute bronchiolitis.

Study design In this South-East Norwegian cohort study, 308 children hospitalized with moderate to severe acute bronchiolitis in infancy in 2010-2011 were compared with 223 healthy controls included in 2012 by measuring morning salivary cortisol levels at inclusion and at 2 years of age. Samples were collected shortly after awakening after 6 AM. The influences of age, sex, and acute bronchiolitis were assessed by regression analysis.

Results In infancy, cortisol values were higher in acute bronchiolitis, with an age- and sex-adjusted weighted mean group difference of 13.9 nmol/L (95% CI 8.1-19.7; $P < .0001$). The median level in reference group was 23.7 nmol/L (95% CI 9.7-119.6). At 2 years of age, sex but not inclusion groups differed, with significantly higher values in girls. The weighted mean of all boys' cortisol levels was 32.4 nmol/L, (95% CI 30.5-34.3), and all girls' levels were 36.9 nmol/L (95% CI 34.7-39.2; $P < .003$).

Conclusions Salivary cortisol levels were higher at 2 years of age than in infancy in the reference group, were higher in girls than in boys at 2 years of age, and were higher in infants at the time of acute bronchiolitis than in healthy infants. *(J Pediatr 2017;184:193-8).*

Trial registration ClinicalTrials.gov: NCT00817466

Cortisol levels normally show a circadian rhythm with physiologically increased levels in the morning, with an additional cortisol awakening response.[1,2] Cortisol levels can be analyzed in blood, urine, and saliva.[3-5] Salivary samples are noninvasive, and do not induce the trauma, stress, and potentially higher cortisol compared with serum sampling.[6] However, a potential shortcoming of salivary measurement is the issue of spot sampling of a biomarker with known diurnal variation.[7] There are few reports on reference values of morning salivary cortisol levels in infants and toddlers, reflecting the biologically active, free fraction of serum cortisol.[8-10]

Reduced morning cortisol has been associated with allergic diseases such as asthma and allergic rhinitis in young and older children, pointing to an involvement of adrenocortical function.[11-14]

Links between stress, cortisol levels, and asthma in early childhood or later asthma development have been proposed,[14-16] and we recently showed that being hospitalized for acute bronchiolitis in infancy increased the risk for reduced health-related quality of life.[17] However, investigation of possible causal associations between infant salivary cortisol levels and later asthma requires relevant reference values of morning salivary cortisol levels in early childhood.

Our primary aim was to describe reference values for morning salivary cortisol levels during infancy and at 2 years of age. Second, we sought to investigate whether age, sex, or acute moderate to severe bronchiolitis in infancy influenced morning salivary cortisol levels.

Methods

The present study included 531 children with at least 1 (total 762) salivary cortisol level measurement in infancy, when they were recruited into the study and/or at the 2-year follow-up (**Figure 1**; available at www.jpeds.com). The source population included 404 infants hospitalized with moderate to severe acute bronchiolitis in 8 pediatric departments of southeast Norway. Additionally, 240 infants were recruited by postal invitation to 3000 randomly selected children 0-12 months of age from the municipalities of Oslo and Fredrikstad[17] from March 22, 2012 to July 2, 2012, who were included in the Bronchiolitis ALL SE-Norway study

RSV Respiratory syncytial virus

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(hereafter referred to as the Bronchiolitis study).[18] As previously reported, respiratory syncytial virus (RSV) was identified in 83% and human rhinovirus in 34% of participants; 44% received oxygen therapy and 7.4% received ventilatory support.[18,19] The follow-up investigation at 2 years, performed from September 27, 2011, to December 14, 2011, September 11, 2012 to December 18, 2012, and October 7, 2013 to January 22, 2014, was attended by 499 of the initial 644 infants (77.5%).

Inclusion criteria for all infants were age 0-12 months, and for inclusion into the Bronchiolitis study, moderate to severe bronchiolitis, with a clinical score of at least 4 on a scale from 0 to 10 (most severe).[18] Exclusion criteria for all infants were severe underlying disease, and for the Bronchiolitis study, more than one episode of either bronchopulmonary obstruction or cough lasting for longer than 4 weeks before recruitment.

Procedures

Clinical investigations, morning cortisol sampling, and parental structured interviews were conducted at inclusion and at 2 years of age.

The study was approved by the Regional Committee for Medical and Health Research Ethics and The Norwegian Data Protection Authority and was registered in the Norwegian bio bank registry as well as ClinicalTrials.gov number, NCT00817466. Written informed consent was obtained from caregivers of all children.

Saliva Sampling

Parents were instructed to sample saliva in the morning as soon as possible after the child's awakening (after 6:00 a.m.) and before the children's first meal. Two small (0.7 × 1.8 mm), tasteless, arrowhead-shaped Sorbette (hydrocellulose, Salimetrics Europe Ltd, Suffolk, UK) microsponges attached to plastic applicator shafts were inserted into the child's mouth, preferably under the tongue, and kept there for a total of 60-90 seconds, until the microsponges were soaked with saliva.[20] The salivary samples in their respective standard containers were brought to the investigation site, and thereafter frozen at −86°C until transferal to Karolinska Institutet, Stockholm, for analysis. Radioimmunoassay was performed according to the manufacturer's instructions using kits from Cisbio Bioassays (Codolet, France) with monoclonal rabbit antibodies binding cortisol. For further description, see the **Appendix** (available at www.jpeds.com). The assay is standardized against the reference method, mass spectrometry.

Main Outcome

Reference values were defined as salivary cortisol levels (nmol/L) ranging from the 5th to the 95th percentile in infancy (at inclusion) and at 2 years of age. For comparison with other studies, geometric means were estimated and reported. Secondary outcomes for assessing potential influence of age, sex, and acute bronchiolitis were weighted mean salivary cortisol levels (nmol/L) with 95% CI.

Statistical Analyses

Background characteristics are given as means with SD, mean with minimum and maximum, or numbers with percentages, as appropriate. Neither morning salivary cortisol levels nor their natural logarithms were normally distributed. Percentiles including 95% CI for the 5th and 95th percentiles were used for estimating reference values.

To assess the potential impact of age, sex, and hospitalization for acute bronchiolitis on morning salivary cortisol levels, associations with cortisol were examined by the Huber M method of regression analysis,[21] whereas associations between dichotomous variables were analyzed by Pearson χ^2 test. Weighted means were calculated by Huber M regression methods, applying groups as categorical values, and estimating intercepts as the weighted mean. The significance level was set at .05. Interaction between age, sex, and morning salivary cortisol was tested by 2-way robust regression. Percentile analyses and robust regression analyses were done with NCSS 2007 (NCSS Statistical Software, Kaysville, Utah); otherwise, IBM SPSS (SPSS Inc, Chicago, Illinois) version 22.

Results

Salivary samples were obtained from January 15, 2010, to May 20, 2011, from 383 infants at a mean age of 5.1 months (range 0.2-13.4) and from 379 children at a mean age of 24.2 months (range 17.2-34.7; **Table I**), with samples at both time points in 231 children and on 1 occasion in the remaining 300 children (**Figure 1**). Background characteristics were similar between children from the reference group and bronchiolitis group with respect to sex, age at 2 years, parental asthma, ethnicity, and breast feeding, but significantly different with respect to weight and length, parental education, and use of inhaled corticosteroids (**Table I**). No interaction was found between age, sex, or morning salivary cortisol at the 2 time points.

Morning salivary cortisol ranged from 1.9 to 691.4 nmol/L in infancy and from 2.5 to 189.0 nmol/L at 2 years of age.

Reference Values

In infancy, the reference group had a geometric mean of 26.8 (95% CI 24.0-30.0) nmol/L with the reference values given by percentiles (**Table II**). The bronchiolitis group had significantly higher cortisol values (**Figures 2-4**; **Figures 2** and **3** available at www.jpeds.com), with a geometric mean of 37.0 nmol/L (95% CI 33.0-41.4) and a median of 39.9 nmol/L.

At 2 years of age, the weighted mean cortisol values were similar in the control and bronchiolitis groups. Reference values were therefore based on values including all children (**Table II**; **Figures 5** and **6**, available at www.jpeds.com), with a geometric mean of 32.1 nmol/L (95% CI 30.4-33.9).

Cortisol levels were above 3 SD in 1.5% and in 1.9% of the children at inclusion and at 2 years of age, respectively. By robust regression, we found no association or individual