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Changes in salivary cortisol levels as a prognostic predictor in children with anorexia nervosa

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ABSTRACT

This study investigated the hypothalamus–pituitary–adrenal (HPA) axis activity in children with anorexia nervosa (AN) before and after inpatient treatment. Salivary cortisol levels were measured to ascertain whether changes in the HPA axis activity following therapeutic intervention could be applicable as a prognostic predictor. This study comprised 21 females with AN and 22 control subjects. Saliva was collected at 2-hour intervals from 9 a.m. to 7 p.m. before and after inpatient treatment. The concentrations for areas under the curve (AUC) were compared with physical parameters, eating attitude score, profile of mood states (POMS), and prognostic factors. Mean salivary cortisol levels at all points and mean AUC cortisol levels in subjects with AN before therapy were significantly higher than those in controls, but returned to control levels after inpatient treatment. Higher AUC cortisol levels were associated with lower standard deviation for weight in AN. A significant positive correlation between the AUC cortisol level and POMS subscale of "Fatigue" was apparent in the control group, but not in the AN group. The increased change values of AUC cortisol level before and after inpatient treatment correlated with increased body weight gain ratio just after treatment, but not with the ratio after one year. The present study indicated that HPA axis activity could reflect severity of illness, but did not show an accurate neuroendocrine response for mood states. Changes in HPA axis activity following treatment could therefore be used to predict prognosis and particularly in the short term.

1. Introduction

Anorexia nervosa (AN) is a disturbance of eating behavior or weight-control behavior, which commonly occurs during adolescence in girls and may result in a clinically significant impairment of physical and psychosocial function (Anderluh et al., 2003; Fairburn and Harrison, 2003). Loss of appetite, distortion of body image, overevaluation of shape and weight, obsessive fears of being fat, and amenorrhea characterize this disorder. The recent increase in childhood AN has underlined the important adverse consequences that such a trend may have on the individual's physical and mental development, and on their risk of chronic diseases in adulthood.

Increased hypothalamus–pituitary–adrenal (HPA) axis activity has been postulated to influence the onset and course of AN in adulthood (Licinio et al., 1996). The endpoint of HPA axis activation is the release of the glucocorticosteroid cortisol from the adrenal glands. Several studies showed increased salivary or serum cortisol levels in the acute phase of adult AN (dos Santos et al., 2007; Gold et al., 1986; Putignano et al., 2001), and suggested that this condition may occur as a consequence of chronic food restriction, as a biological adaptation to starvation. High cortisol levels decrease gonadotropin-releasing hormone (GnRH) secretion and luteinizing hormone (LH) pulse frequency (Barbarino et al., 1989). The secretion of GnRH from hypothalamus controls the release and synthesis of the pituitary gonadotropins including follicle stimulating hormone (FSH) and LH, which are both important in regulating the menstrual cycle. FSH grows the ovarian follicles and LH induces follicular rupture and sustains the corpus luteum, while both FSH and LH induce proliferation and differentiation of the uterine endometrium. An inverse association has been noted between cortisol level and menstrual frequency (Laughlin et al., 1998). Cortisol excess also inhibits osteoblasts and stimulates osteoclasts, impairs calcium absorption from the gut (Misra and Klibanski, 2009), and is associated with low bone mineral density in AN (Misra et al., 2008). Consequently, measuring baseline levels of neuroendocrine modulators/hormones including ghrelin, leptin, peptideYY, and cortisol was proposed as a way to predict overall recovery in AN (Lawson et al., 2010; Misra et al., 2006a).

Salivary cortisol is also routinely used as a biomarker of psychological stress and related mental or physical diseases (Hellhammer et al., 2009; Sjörs et al., 2010). Depressed adults show increased total cortisol secretion and a flattened diurnal rhythm is characteristic

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of HPA axis hyperactivity (Goodyer et al., 2001). Young people at familial risk of depression, but with no personal history of mood disorder, show higher cortisol secretion compared to controls with no familial history of depression, indicating that elevated cortisol secretion is a prospective predictor of major depression and may serve as a vulnerability marker (Mannie et al., 2007). Lawson et al. (2009) reported a positive association between baseline cortisol levels and anxiety or depression scales in AN, suggesting that hypercortisolemia is a potential mediator of mood disturbance in these patients.

The relationship between HPA axis activity and physical or psychological condition in childhood AN (Zonnevylle-Bender et al., 2005) has not been investigated. It also remains unclear whether longitudinal variations in such activity would predict prognosis in childhood AN. To address these research questions, we measured changes in salivary cortisol level from baseline before and after inpatient treatment to determine the prognostic significance of those levels as predictors for recovery in childhood AN.

2. Methods

2.1. Participants

The patients were 21 female children with AN who fulfilled the Great Ormond Street (GOS) criteria (Nicholls et al., 2000), developed for childhood AN. They were diagnosed and received medical care at the Department of Pediatrics, Kurume University School of Medicine, Japan, from 2004 to 2010. Mean age and body mass index (BMI) at onset were 14.4 ± 1.4 years and 13.0 ± 1.5 kg/m^2 (mean $\pm$ SD), respectively. All patients were of the restrictive type. The inpatient treatment program consisted of two phases, with the first phase aimed at weight restoration and normalization of eating, based on standard behavioral therapy principles. After gaining a basic understanding of these principles, that is, a behaviorally oriented token economy, each patient discussed target weight as the treatment goal. The behavioral regimens were designated individually and as the patient gained weight, additional hospital privileges were granted. Each individual's diet began at 500–1000 cal/day. This calorie intake was increased gradually as the patient achieved treatment gains. Severely anorexic patients received liquid food supplements by nasogastric feeding. In the second phase, patients were instructed on psychological therapies, focusing on issues of self-image, self-evaluation, and family function. Parental counseling was regularly conducted during the patient's hospitalization. Three elementary-school girls had not commenced menstruation and the remaining girl showed secondary amenorrhea throughout the treatment. One patient took selective serotonin reuptake inhibitor (SSRI) medication during the treatment. The inpatient treatment raised the mean BMI to 15.5 ± 1.5. All patients then received further nutritional and psychological counseling through the outpatient clinic. Some AN patients recovered from amenorrhea during the outpatient observation period. Twenty-two age-matched female control subjects who had no history of eating problems, other psychiatric disease, organic disease, psychiatric treatment, or endocrine disease were also enrolled. Their mean BMI of 19.3 ± 2.1 was significantly different from that of the AN group. Mean age among the control group was 14.0 ± 1.2 years old.

The Ethics Committee of Kurume University School of Medicine approved the study protocol and written informed consent was obtained from each patient and control.

2.2. Salivary cortisol measurement

Salivary samples were taken six times a day at 2-hour intervals from 9 a.m. to 7 p.m. (0900 h, 1100 h, 1300 h, 1500 h, 1700 h, and 1900 h). The samples were collected from the AN group at the hospital and at home for the control group. Control subjects were requested to stay at home on weekends for saliva sampling. Saliva was not collected in the control group during menstruation. For the AN group, diurnal salivary sampling was performed twice during the inpatient treatment, at the beginning and end; however, saliva was not collected within two weeks after admission until the patient was acclimatized to their new environment. The second sampling was not collected in one patient, because she refused to participate. The mean interval between first and second samplings was 96.1 ± 53.9 days. All subjects were instructed not to collect saliva within 30 min after eating, drinking, walking, and teeth brushing. To avoid the possible influence of food intake on cortisol level, participants were asked to eat meals at regular times (breakfast at about 0800 h, lunch at about 1200 h and dinner at 1800 h). Salivary sampling is a well-established technique for cortisol measurement in adults and children. Saliva was collected into a commercially available device using a cotton swab that was chewed for 3 min, and then inserted into a double-chamber plastic test tube. Saliva samples were centrifuged at 4 °C and stored at −20 to −80 °C until required for assay. Salivary cortisol was measured by an enzyme-linked immunosorbent assay (ELISA) (SLV-2930, DRG International, USA) as previously described (Brydon et al., 2009). The limit of sensitivity was 0.53 ng/ml salivary cortisol and the intra-and inter assay error for salivary cortisol was 2.61% and 3.63%, respectively.

2.3. Psychological assessments

To evaluate whether eating behavior and mood disturbance were associated with cortisol level, all participants were rated according to the Eating Attitudes Test (EAT-40) and Short form of the Profile of Moods Scale (POMS). EAT-40 has been widely applied as an index of AN symptoms (Alvarez-Rayón et al., 2004), while the POMS assessment is an excellent measure of fluctuating affective mood state. The short form of POMS consists of 30 items, describing six moods: Tension–Anxiety, Depression–Dejection, Anger–Hostility, Vigor, Fatigue, and Confusion. Each original POMS score was converted to a T-score (McNair et al., 1971).

2.4. Prognostic factors

We evaluated body weight gain and resumption of menstruation during the recovery process of AN as prognostic factors. Body weight before treatment, just after inpatient treatment, and one year after treatment were described as standard deviation (SD) using cross-sectional growth curves for Japanese girls. Body weight gain ratio at the end of inpatient treatment was calculated by subtracting the SD at the beginning of treatment from that attained just after inpatient treatment. Body weight gain ratio at one year after treatment was calculated by subtracting the SD at the beginning of treatment from that at one year after treatment. Resumption of menstruation was assessed one year after inpatient treatment.

2.5. Data analyses

Mean salivary cortisol levels at each point were compared among AN patients before inpatient treatment, AN patients after the inpatient treatment, and controls. Further, each subject's diurnal cortisol data was expressed as the areas under curve (AUC) (Fekedulegn et al., 2007). The mean AUC were also compared among the three groups. Mean EAT-40 score and each subscale POMS score were compared with those of controls. The baseline AUC cortisol level (AUC cortisol level before inpatient treatment) was used to assess the correlation with BMI, SD for body weight, EAT-40 score, and each subscale POMS score. The change value of AUC cortisol level following therapeutic intervention was calculated by subtracting the AUC cortisol level after inpatient treatment from the level before therapy. The change value of AUC cortisol level was used to assess correlation with prognostic factors. The mean baseline and the change value of AUC cortisol levels were compared between AN patients with and