Cortisol exposure, cognition and clinical course of bipolar disorder

Spijker, A.

6. Long-term cortisol in Bipolar Disorder: Associations with age of onset and psychiatric co-morbidity

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Abstract

Introduction:

Dysregulation of the hypothalamic-pituitary-adrenal (HPA-)axis is hypothesized to play a role in the pathogenesis of bipolar disorder (BD). Conflicting results have been reported when saliva or serum was used to measure cortisol levels. A recently developed method is to measure cortisol in scalp hair, with one cm of scalp hair representing one month. We studied whether there are differences in long-term hair cortisol levels between BD patients and healthy individuals and whether there are associations between hair cortisol and disease characteristics.

Methods

Hair samples were collected in 100 BD patients and 195 healthy controls. Long-term cortisol levels were determined in 3 cm hair segments. Saliva samples were collected on two consecutive evenings. Documented disease characteristics were disease state, age of onset and psychiatric co-morbidity.

Results

Hair cortisol levels were not statistically different in BD patients compared to healthy controls ($p=0.233$) and were not associated with the disease state at the moment of sample collection ($p=0.978$). In the subgroup of patients with age of onset ≥30 years, hair cortisol levels were significantly elevated compared to the subgroup with age of onset <30 years and to healthy controls ($p=0.004$). Psychiatric co-morbidity was associated with elevated cortisol levels (44.87 versus 31.41 pg/mg hair; $p=0.021$), with the exclusion of panic disorder, which was associated with decreased cortisol levels (22.13 versus 34.67 pg/mg hair; $p=0.019$).

Conclusions

Elevated long-term cortisol levels might play a role in a subgroup of patients with BD. There may be differences in pathogenesis of younger and older onset BD suggesting two different disease entities.

Introduction

Cortisol, the main glucocorticoid in humans, is released in response to stress as part of the hypothalamic-pituitary-adrenal (HPA) axis that affects mineralocorticoid and glucocorticoid receptors throughout the brain. Dysregulation of the HPA-axis is known to occur in several psychiatric disorders, such as anxiety disorder (e.g. post traumatic stress disorder) (1) and mood disorders including bipolar disorder (BD) (2-7). In addition, depression and mania are common psychiatric symptoms seen in patients treated with corticosteroids or suffering from Cushing's Syndrome, a disorder caused by cortisol excess (8, 9). Dysregulation of the HPA-axis in BD is also supported by several studies that have found more non-suppression after dexamethasone (dex) administration and increased cortisol secretion after corticotrophin releasing hormone (CRH) in patients with BD (10, 11). However, conflicting data have been published concerning HPA-axis dysfunction during episodes and during euthymic phases of the disease. Furthermore, there are only a few studies reporting dexamethasone suppression tests (DSTs) or combined dex/CRH tests in patients with bipolar disorder (10, 11).

In the majority of previous studies salivary or serum cortisol levels have been used to evaluate HPA-axis functioning. However, these studies yielded conflicting findings. Some studies reported no differences in basal salivary or serum cortisol levels in patients with BD (12-15), whereas others described elevated cortisol levels (16, 17). These conflicting findings might be the result of methodological differences between the studies. Some studies reported cortisol levels in patients in remission, while others measured cortisol levels during active depression or mania. Furthermore, there is a large discrepancy in the methods used to evaluate cortisol levels in BD patients. Some studies evaluated the cortisol awakening response, whereas others investigated diurnal rhythms of cortisol secretion or cortisol levels at single time points. Cortisol is secreted in a circadian rhythm and with a pulsatile pattern, which complicates the interpretation of serum and salivary cortisol levels. Furthermore, in blood, only 10% of the circulating cortisol is free, 75% is bound to cortisol binding globulin (CBG) and 10% to albumin (18). In most studies describing serum cortisol levels, the concentration of CBG is not taken into account. Therefore the concentrations of unbound, biologically active, cortisol are not known. In addition, since cortisol is a stress hormone, acute psychological or physical stress will also influence serum and saliva cortisol levels. A recently developed method to measure cortisol levels in scalp hair is a feasible method to determine long-term cortisol levels and appears to yield a reliable estimate of long-term HPA-axis activity (19, 20). Since hair grows with an average rate of 1 cm per month, a hair segment of e.g. 3 cm would reflect mean cortisol levels over a period of approximately 3 months. This long-term cortisol measurement is therefore not influenced by the time of sample collection or acute

stress due to daily circumstances or the research setting. Steudte et al. (21) used hair cortisol levels to measure HPA-axis activity in generalized anxiety disorder (GAD) and found decreased cortisol levels in hair of patients with GAD, but no differences in salivary cortisol levels between GAD patients and healthy controls (22). This suggests that hair cortisol levels may reflect the chronic cortisol exposure, whereas the results found with saliva or serum cortisol levels might also include acute responses to the measurement circumstances. Several other studies have shown that hair cortisol is indeed a marker of long-term cortisol exposure (19, 23-26).

The aim of this study was to explore the role of long-term endogenous cortisol exposure by comparing long-term cortisol levels in BD patients with healthy controls using cortisol measurements in scalp hair. We aim to obtain insight in the long-term assessment of cortisol in patients with bipolar disorder, since chronic subtle changes in HPA-axis functioning appears to be involved in the pathophysiological processes leading to mood episodes. We hypothesized that hair cortisol levels are higher in BD patients compared to healthy individuals. In addition, we explored the relation between cortisol levels in hair and clinical course of the disease, with characteristics like disease state, age of onset, and psychiatric co-morbidity. Furthermore, we measured saliva cortisol levels in order to compare the observed findings of hair cortisol.

Materials and methods

Study design

This is a cross-sectional study involving outpatients with BD. The study was approved by the local medical ethics committee and is carried out in accordance with the declaration of Helsinki. After complete description of the study to the participants, all participants gave informed consent.

Participants

Bipolar Disorder patients

Patients with BD (type I, type II and Not Otherwise Specified) included in our previous study (27) concerning the role of glucocorticoid and mineralocorticoid receptor polymorphisms were asked to participate and the first 100 eligible patients were included in this study. Patients were eligible for inclusion if they had not been using glucocorticoids

in the six months prior to hair sample collection and if they had sufficient hair growth at the posterior vertex. Detailed description of the assessment methods of the patients has been described elsewhere (27, 28). In brief, participants were interviewed by trained psychologist to collect socio-demographic data and disease characteristics. Diagnoses of BD and psychiatric co-morbidities were based on DSM-IV criteria and were assessed with a standardized diagnostic interview developed by Sheehan et al. (29) using the Dutch version of the MINI International Neuropsychiatric Interview Plus (version 5.00-R; MINI-PLUS) (30). The Questionnaire for Bipolar Illness, Dutch translation (31, 32) was used to specify subtypes of BD, its course over time and detailed information about age of onset of first symptoms regarding hypomanic, manic and depressive episodes. The Questionnaire for Bipolar Illness, Dutch translation is a widely used questionnaire, developed by the National Institute of Mental Health. In addition, detailed information was gathered to define whether patients suffered from depression, mania or a combined episode during the three months prior to hair sample collection (this timeframe corresponds with cortisol measurements in 3 cm hair segments). Based on this information**, disease** state was categorized as stable disease, depressive episode, manic episode or mixed episode. **Data** on age of onset were present in 84 patients and data concerning psychiatric co-morbidities were present in 99 patients.

Healthy controls

A group of 195 healthy controls were used as control group. Detailed information of this study group has been reported previously (19). Both patients and healthy controls filled out a questionnaire concerning hair treatment (dyeing/bleaching/permanent waving/straightening), the use of hair products (gel, wax, hair spray) and frequency of hair wash.

Sample collection

In all patients and healthy controls, a lock of hair of approximately 100-150 hairs was cut of from the posterior vertex as close to the scalp as possible. Hair was taped to a paper and stored until preparation. In addition, in 90 patients with BD also saliva samples were collected on two consecutive evenings at 2200h. Collection at 2200h was chosen since several studies showed that saliva samples collected in the late evening on separate days show the lowest variation in cortisol levels compared to diurnal cortisol measurements and the cortisol awakening response (33-35). This suggests that late evening cortisol levels are slightly less influenced by acute stressors and daily influences than e.g. the cortisol awakening response, and may thereby be a better reflection of basal cortisol status. Participants were instructed not to eat, drink or brush their teeth 30 minutes prior

to the saliva collection. Saliva was collected by spitting into a Salicap plastic tube. Saliva samples were stored at -20°C until analysis. Cortisol levels were measured separately in both saliva samples and we calculated the mean salivary cortisol levels afterwards. We used the mean salivary cortisol levels in the statistical analyses.

Hair sample preparation

Hair samples were measured, weighted and put into separate glass vials. The 3 cm segment most proximal to the scalp was used in the analysis, which corresponds roughly to a period of three months. In the glass vial, hair segments were cut into small pieces and 1 mL of methanol was added to extract cortisol from the hair samples. After overnight incubation for 16 hours at 52°C while gently shaking, the methanol was transferred to another glass vial and was evaporated under a nitrogen stream and the samples were dissolved in 250 µL phosphate buffered saline (pH 8.0).

Cortisol measurement

Saliva samples were thawed and vortexed. After this, saliva samples were centrifuged for 5 minutes to separate the mucins. Cortisol in saliva as well as in the hair extracts was measured using a commercially available salivary ELISA cortisol kit (DRG Instruments GmbH, Marburg, Germany). Cross reactivity of the kit's antibodies with other steroids was reported as follows: Corticosterone (29.00%), Cortisone (3.00%), 11-Deoxycortisol (<1.00%), 17-OH Progesterone (<0.50%), other hormones (<0.10%). Intra-assay variation was below 5% and the inter-assay variation below 8% as stated by the manufacturer. The low end detection limit for this assay is 1.5 nmol/L. To test the recovery of the assay, we created cortisol standards in PBS with concentrations of 5, 10, 20, 40, 80 and 160 nmol/L and measured the recovery in duplicate. We also spiked two hair samples with 20 nmol/L hydrocortisone, to measure recovery when hydrocortisone was dissolved in hair extract. The recovery of 5, 10, 20, 40, 80 and 160 nmol/L cortisol standards from PBS was 96.0%, 94.0%, 94.0%, 95.0%, 96.8% and 89.9% respectively. When hydrocortisone was added to hair extracts, the mean recovery was 101.3%.

Statistical analysis

SPSS 17.0 for Windows and GraphPad 5.0 were used for statistical analysis. Differences between group characteristics were tested with Chi-square and Kruskal Wallis tests. After log transformation hair and saliva cortisol levels were normally distributed. Differences in cortisol levels between healthy controls and BD patients and between

disease characteristics of BD patients were tested with ANCOVA and linear regression. The association between saliva cortisol and hair cortisol levels was tested using linear regression analysis. All hair cortisol analyses were adjusted for gender, age, frequency of hair wash, hair treatment (dyeing, bleaching, permanent straightening or waving) and the use of hair products. Saliva cortisol analyses were adjusted for gender and age.

Results

Hair cortisol measurements were completed in 100 BD patients and 195 healthy controls. Saliva cortisol levels were available in 90 BD patients. Group characteristics are shown in Table 1. All BD patients were treated in the outpatient Department of Mood Disorders in The Hague, the Netherlands. BD patients were significantly older than healthy controls and had significantly higher BMI. Furthermore, BD patients washed their hairs less frequently and used less hair products, but dyed/bleached their hairs more often compared to the healthy control group (Table 1).

The number of patients on lithium, antidepressants, antipsychotics and other mood stabilizers are shown in Table 1. There were only 2 (2.0%) BD patients that did not use anti-depressants, antipsychotics, lithium or other mood stabilizers, 30 patients (30.0%) used only 1 type of medication, 31 patients (31.0%) used 2 types of medication and 19 patients (19.0%) used 3 types of medication. There was no correlation between log-transformed saliva cortisol and hair cortisol measurements (b=0.157, p=0.140) (Figure 1).

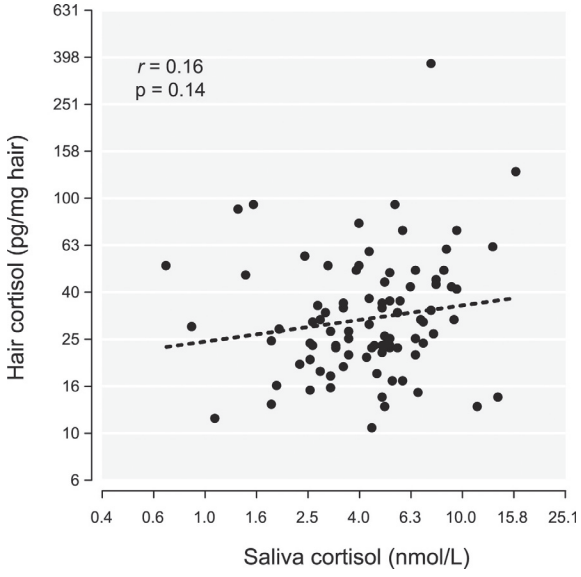
Table 1. Group characteristics

	Bipolar Disorder (n=100)	Healthy Controls (n=195)	p-value
Age (years) – median (range)	52.0 (20-82)	32.0 (18-63)	<0.001
Number of women – n (%)	62 (62.0%)	103 (52.8%)	0.18
BMI (kg/^{2}) – median (IQR)	25.3 (23.5-28.0)	23.7 (21.7-26.5)	<0.001
Frequency of hair wash – n (%)			
≤ 2 times/week	53 (53.0%)	50 (25.6%)	
≥ 3 times/week	47 (47.0%)	143 (73.3%)	<0.001
Hair treatment*	40 (40.0%)	43 (22.1%)	0.001
Use of hair products**	32 (32.0%)	90 (46.2%)	0.018
Age of onset Bipolar Disorder			
median (IQR)	21.0 (15.5-35.0)		
< 30 years of age – n (%)	55 (55%)		
> 30 years of age – n (%)	29 (29%)		
One or more psychiatric co morbidities – n (%)	42 (42.0%)		
Co morbid panic disorder	14 (14.0%)		
Co morbid anxiety disorder	16 (16.0%)		
Co morbid agoraphobia	21 (21.0%)		
Co morbid pain disorder	1 (1.0%)		
Co morbid somatoform disorder	2 (2.0%)		
Medication			
Lithium – n (%)	68 (68.0%)		
Other mood stabilizers – n (%)	17 (17.0%)		
Antidepressants – n (%)	31 (31.0%)		
Antipsychotics – n (%)	34 (34.0%)		
Disease state in the period covered by the hair sample			
Stable disease– n (%)	43 (43.0%)		
Depressive episode – n (%)	29 (29.0%)		
Manic episode – n (%)	3 (3.0%)		
Mixed episode – n (%)	8 (8.0%)		

* *Hair treatment: dyeing, bleaching, permanent waving, permanent straightening of hairs*

*** Hair products: includes hair spray, wax, mouse and gel and other not wash-related hair products.*

Figure 1. *Relationship between the mean cortisol level in two evening saliva samples (collected at 22.00h) and the hair cortisol levels on logarithmic scales. The Pearson's correlation coefficient and its accompanying p-value are given with a regression line.*

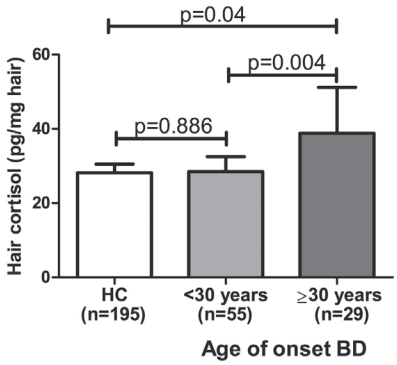


Hair cortisol

There were no correlations between hair cortisol levels and age in healthy controls (r=0.062, p=0.39) or BD patients (r=-0.047, p=0.64). There were also no differences in hair cortisol levels between men and women in healthy controls (p=0.87) or BD patients (p=0.12) and hair cortisol levels did not correlate with BMI in BD patients (r=0.163, p=0.11) or in healthy controls (r=0.042, p=0.59). Hair cortisol levels in the total group of BD patients were not different from hair cortisol levels in healthy controls (31.84 pg/mg hair (95% Confidence interval (CI): 28.38 – 35.81) versus 28.18 pg/mg hair (95% CI: 25.94 – 30.62); p=0.233, adjusted for gender, age, hair treatment, hair products and frequency of hair wash). Since there was variability in the state of disease at the moment of sample collection, the group of BD patients was split up into groups with a stable period (n=43), depressive episode (n=29), manic episode (n=3) or combined episode (n=8) in the 3 months prior to sample collection. There were no significant differences between hair cortisol levels in the groups with various disease states in the three months prior to hair sample collection (unadjusted p=0.868; adjusted for gender, age, hair treatment, hair products and frequency of hair wash p=0.978). In addition, there was no effect of lithium (p=0.357), other mood stabilizers (p=0.388), antidepressants (p=0.816) or antipsychotics (p=0.278) on cortisol levels.

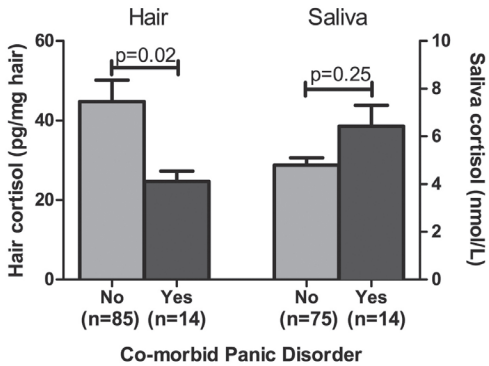
We found no significant correlation between age of onset of BD (age at which the first episode of (hypo)mania or depression presented) and hair cortisol levels (b=0.156, p=0.179). . However, when assessing the distribution of hair cortisol throughout the different age of onset groups divided in decades, we found significantly elevated hair cortisol levels in the group of patients with their first depression or mania ≥ 30 years of age compared to the group of patients with their first depression or mania before 30 years of age (p=0.004) (Figure 2). Hair cortisol levels in the group with onset of BD < 30 years of age were similar to those in the healthy controls (p=0.886, adjusted for gender, age, hair treatment, hair products and frequency of hair wash) (Figure 2). There were no differences in use of lithium (p=0.536), other mood stabilizers (p=0.384), antidepressants (p=0.397) and antipsychotics (p=0.278) between the group of patients with an older age of onset of BD compared to the group of patients with an age of onset <30 years. Furthermore, there was no correlation between duration of the disease and hair cortisol levels (r=-0.105, p=0.34).

Figure 2. *Hair cortisol levels in healthy controls, bipolar disorder patients with younger and older age of onset. HC, healthy controls; p-values are adjusted for age, gender, use of hair products, hair treatment and frequency of hair wash. Error bars represent SEM.*



Interestingly, in patients with co-morbid panic disorder (n=14) hair cortisol levels were significantly lower than in BD patients without panic disorder (22.13 versus 34.67 pg/mg hair, p=0.019, adjusted for gender, age, hair treatment, hair products and frequency of hair wash) (Figure 3). These hair cortisol levels in patients with co-morbid panic disorder were even lower than hair cortisol levels in healthy individuals (p=0.05, adjusted for gender, age, hair treatment, hair products and frequency of hair wash). Furthermore, we found elevated hair cortisol levels in the group of BD patients with other psychiatric co-morbidities (e.g. anxiety disorders, agoraphobia, pain disorder, somatoform disorder) compared to the BD patients without psychiatric co-morbidities (44.87 pg/mg hair (95% CI: 34.36-58.61) versus 31.41 pg/mg hair (95% CI: 26.06-37.76) in patients without co-morbidities, p=0.021). When patients with co-morbid panic disorder were included in the group of total co-morbidities, there was no significant difference in hair cortisol levels between BD patients with and without psychiatric co-morbidities anymore (34.67 versus 31.33 pg/mg, p=0.448). Additional adjustment for BMI did not change any of the results.

Figure 3. *Hair cortisol and salivary cortisol levels in bipolar disorder patients with and without co-morbid panic disorder. Hair cortisol analyses are adjusted for age, gender, use of hair products, hair treatment and frequency of hair wash. Saliva cortisol analyses are adjusted for gender and age. Error bars represent SEM.*



Saliva cortisol

There were no significant differences in salivary cortisol levels between BD patients with a depressive episode, manic episode or stable disease at the moment of saliva collection (p=0.304). In addition, we found no differences in salivary cortisol levels between age of onset of disease before the age of 30 years and after the age of 30 years (p=0.155). In contrast to the results with hair cortisol levels, co-morbid panic disorder was associated with a trend towards increased salivary cortisol levels (5.5 (95% CI: 4.0-7.5) nmol/L versus 4.1 (95% CI: 3.6-4.7) nmol/L, unadjusted p=0.094). However, this was not statistically significant after adjustment for gender and age (p=0.245) (Figure 3). Furthermore, we found no differences between the group of BD patients with psychiatric co-morbidities (panic disorder excluded) and without psychiatric co-morbidity (p=0.562). Also, when panic disorder was included in the total group of psychiatric co-morbidities, there were no significant differences in salivary cortisol levels between the BD patients with and without co-morbidities (p=0.306). An overview of all results for hair and salivary cortisol is given in Table 2.

Table 2. Overview of the hair cortisol and saliva cortisol results of disease characteristics in patients with Bipolar Disorder

	Hair cortisol				Salivary cortisol			
	Crude b	p	Adjusted b	Adjusted p	Crude b	p	Adjusted b	Adjusted p
Age of onset Bipolar Disorder (<30 versus ≥ 30 years)	0.249	*0.022*	0.335	*0.004*	0.166	0.146	0.163	0.155
Co morbid panic disorder	-0.211	*0.036*	-0.243	*0.019*	0.187	0.094	0.122	0.245
Psychiatric co morbidity (excl. panic disorder)	0.227	*0.036*	0.253	*0.021*	0.020	0.865	0.068	0.562
Psychiatric co morbidity	0.087	0.389	0.078	0.448	0.153	0.329	0.142	0.306

The Beta was calculated with linear regression. Hair cortisol analyses were adjusted for age, gender, use of hair products, hair treatment and frequency of hair wash. Saliva cortisol analyses were adjusted for gender and age

Discussion

In this study, we measured long-term hair cortisol levels of patients with bipolar disorder and healthy individuals. In addition, we measured evening salivary cortisol levels in bipolar disorder patients. The main finding of this study is the elevated hair cortisol level in patients with older age of onset BD compared to the group with a younger age of onset and healthy controls. We also found that BD patients with co-morbid psychiatric disorders (excluding panic disorder) had higher hair cortisol levels than patients with only BD. Interestingly, in patients with co-morbid panic disorder, hair cortisol levels were significantly decreased, whereas saliva cortisol levels were slightly elevated. No further correlations between hair and salivary cortisol measurements were found.

This is the first study that measured long-term hair cortisol levels of patients with BD. Several studies have measured cortisol levels in serum and saliva and results have been inconclusive with reports of increased (16, 17) and normal cortisol levels (12-15) compared to healthy individuals. Serum and saliva cortisol measurements reflect cortisol levels at one time point and are influenced by the pulsatile pattern and circadian rhythm of cortisol secretion, as well as by acute stress due to daily circumstances. These limitations do not apply to hair cortisol measurements which makes hair cortisol a better marker of HPA-axis functioning on the long-term. Recently, two studies measured hair cortisol levels and related them to mood disorders (36, 37). Dettenborn et al. showed that hair cortisol levels were elevated in medicated patients with major depression (37) and Dowlati et al. showed that there was no difference in hair cortisol levels between depressed and non-depressed patients with coronary artery disease (36). However, hair cortisol levels of both depressed and non-depressed patients with coronary artery disease were higher than those in healthy controls. The possible psychological stress associated with suffering from coronary artery disease and the presence of coronary artery disease itself could have abolished the differences in hair cortisol between depressed and non-depressed patients, resulting in different results than the observations of Dettenborn et al. Since both studies only included patients with major depression and not BD, it is difficult to compare their results to our findings.

In our study, we found that patients with younger (<30 years of age) and older (≥30 years of age) onset BD clearly differed in their mean hair cortisol levels. Our finding of elevated hair cortisol levels in BD patients with older age of onset supports the hypothesis of dysfunction and hyperactivity of the HPA-axis which has been described in BD patients and their offspring (15, 38-43). However, cortisol levels in patients with earlier age of onset BD were comparable to hair cortisol levels in healthy individuals. In the past decade, several studies have found that there might be differences in disease characteristics and pathogenesis of early and late onset BD. Early onset of BD seems to be associated with

greater severity and a poorer long term outcome (44, 45). In addition, several genetic differences have been described between early and late onset BD (46, 47). Differences in cortisol levels and HPA-axis functioning between subtypes of BD based on age of onset have not been studied previously. Our hair cortisol data suggest that elevated cortisol levels may play a role in late onset BD, but not in early onset BD. This suggests that BD onset at younger age may be less influenced by dysregulation of the HPA-axis, but more by other mechanisms e.g. changing regulation of sex hormones or differences in genetics of the disease. Later onset of the disease might be influenced by dysregulation of the HPA-axis through e.g. stressful life events, which are thought to be a trigger for manifestation of the disease (48). Furthermore, it can also be hypothesized, that long-term elevations in cortisol affect proneness for mood disorders by neuronal damage in the brain (49), which may lead to a higher risk of developing mood disorders. As seen in patients with endogenous hypercortisolism, psychopathology continues even after cure (8, 9), suggesting that long-term elevations in cortisol can have irreversible effects on the brain, resulting in mood disorders.

We did not observe these differences in age of onset with saliva cortisol, suggesting that saliva cortisol may be an acute marker of cortisol rather than an estimate of the HPA-axis activity on the long-term. It supports the additional value of measuring hair cortisol levels. Furthermore, we found no differences in hair cortisol levels between patients in different disease states. The same applied to the observed findings of salivary cortisol in relation to BD, which is consistent with several other studies (15, 16). This suggests that the increased HPA-axis activity may be a trait phenomenon rather than a state phenomenon in the subgroup of patients with older age of onset.

Moreover, we found that psychiatric co-morbidity was associated with elevated hair cortisol levels within the group of BD patients. With respect to the different types of co-morbidities, we found significantly lower cortisol levels in BD patients with co-morbid panic disorder. In contrast to this, evening saliva cortisol levels tended to be higher in this group. Our study is the first study that describes both hair and saliva cortisol levels in patients with panic disorder. These data again suggest that hair cortisol levels may reflect cortisol levels over a larger time frame (about three months is this study, since we used three cm length of hair), whereas saliva cortisol may provide an estimate of one time point including potential acute effects of the circumstances. Steudte et al. (22) previously demonstrated similar results in a group of patients with generalized anxiety disorder. In this group they found that hair cortisol levels were decreased compared to healthy individuals, whereas there was no difference between saliva samples of

GAD patients and healthy individuals (22). The increased saliva cortisol levels in our BD patients with panic disorder compared to those without co morbid panic disorder, might be explained by more perceived stress in the participants with panic disorder prior to a visit to the research center than BD patients without panic disorder. Reports of saliva, serum and urine cortisol levels in patients with panic disorder have shown conflicting results (50-55), which emphasizes the need for a new reliable measurement of long-term cortisol exposure.

There are several limitations of this study. First, the healthy individuals were significantly younger than the BD patients, which may have affected our findings. However, there was no effect of age on cortisol levels in the control group (19) or in the bipolar disorder group. In addition, other studies reported no effect of age on hair cortisol levels (56, 57), and all analyses were adjusted for age. Second, several studies have shown that other factors, such as suicide attempts and childhood trauma can have a significant effect on the HPA-axis (58, 59). In our study population, there was no significant effect of childhood abuse (sexual, physical and verbal) on hair cortisol levels (data not shown), but we did not collect data concerning suicide attempts. In future studies, other factors that might influence the HPA-axis such as childhood trauma and suicide attempts should be taken into account as well.

Third, our study lacked data concerning smoking status and somatic health. However, we found no differences in hair cortisol levels between smokers and non-smokers in our group of healthy individuals (data not shown), and a recent study of Dettenborn et al (37) did not find differences in hair cortisol levels between smokers and non-smokers as well. Furthermore, our results of possible increased saliva cortisol levels with decreased hair cortisol levels in patients with panic disorder were found in a group of only 14 patients with co-morbid panic disorder in addition to bipolar disorder. The pathogenesis of panic disorder in BD patients might be different from the pathogenesis of panic disorder in patients without BD or another psychiatric disorder. Therefore, these results have to be interpreted carefully and it is not certain whether the results can be extrapolated to patients with panic disorder without other psychiatric disorders. Finally, measurement of cortisol in scalp hair is a relatively new and promising method but many details concerning hair growth rate and incorporation of cortisol in hair are still unknown.

Despite these limitations, our results support the hypothesis that elevated long-term cortisol levels play a role in a subgroup of patients with bipolar disorder. Since we found that cortisol levels were only elevated in the group of patients with a relatively late age of onset of BD, we hypothesize that there may be differences in pathogenesis among patients with early and late onset BD yielding two different disease entities. Future

research should focus on exploring these subtypes of BD based on age of onset with potentially a differential neuro-endocrine background.

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