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Elevated fingernail cortisol levels in major depressive episodes

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Abstract

Background: Hypercortisolemia is considered a putative biomarker for a depressive episode. There is however uncertainty about the reliability and generalizability of cortisol measurements in major depressive episodes. The use of different specimen types to assess acute cortisol levels in major depression is believed to have contributed to the inconsistencies in the literature. Cortisol extraction from fingernails has recently been proposed as a novel strategy to measure cortisol levels averaged over a period of two weeks. This methodology has not yet been utilised in major depression.

Methods: Cortisol levels reflecting a period of 15 days were measured using fingernails in a group of 26 subjects experiencing a major depressive episode (MDE) and in an age and gender matched group of 45 healthy controls.

Results: Depressed subjects showed significantly higher mean cortisol levels measured in fingernails when compared with control subjects. Higher levels of cortisol were associated with higher depression severity scores, a diagnosis of non-reactive depression, and more prominent melancholic symptoms. Conversely, fatigue was negatively correlated with cortisol levels.

Conclusion: There is elevated cortisol in MDE when assessed using an aggregate measure over two weeks. Alterations in fingernail cortisol correlate with key clinical symptoms and subtypes of depression.

1. Introduction

Hypercortisolaemia is a frequent finding in major depression (Pariante, 2009) and is considered a candidate biomarker potentially able to support the diagnosis or sub-typing of the disorder at biological level. However, there is some inconsistency in this finding, especially when using short-term measurements of cortisol concentrations such as salivary cortisol. This could be due to the susceptibility of these parameters to the influence of state variables, such as the day of the week, time of day or presence of transient extraneous factors liable to alter levels such as stress (Kudielka & Wüst, 2010). Fingernails, an integumentary type of tissue that share several properties with hair, have recently been used and validated for measuring longer-term concentrations of cortisol(Ben Khelil et al., 2011; Izawa et al., 2015). The advantages of this methodology are manifold: it is non-invasive, economical in that repeated sampling can be avoided, and enables the assessment of cumulative cortisol concentrations over an extended period. To date, only one study has used this specimen in the mental health field for this purpose (Warnock et al., 2010) and none in subjects with major depression. Based on the background mentioned above, we designed this study with the objective to (1) compare fingernail cortisol concentrations in depressed subjects and matched healthy controls and (2) assess whether differences in cortisol levels are influenced by clinical variables in subjects with major depression. We hypothesised that subjects with major depressive episodes would be characterised by higher fingernail cortisol levels than healthy controls.

2. Methods

Participants

Participants were recruited in London (UK), Santiago (Chile) and Hong Kong. Depressed participants were recruited via public advertisements (Wise et al., 2016) and from local psychological therapy and secondary care services. All psychometric tools were English or validated Spanish/Chinese versions. Patients met DSM-IV criteria for a major depressive episode (MDE) in the context of either a unipolar or bipolar disorder, diagnosed using the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) and reported no history of psychiatric illness in first degree relatives. Depression severity was assessed with

the Hamilton Depression Rating Scale (HAMD-17; Hamilton, 1967), with only those scoring at clinical severity (≥ 13) included (Cleare et al. 2015). Rating of depressive symptoms across sites was evaluated on an independent set of patients and showed a high inter-rater reliability (Intraclass correlation coefficient=0.96, p=0.004). The Quick Inventory of Depressive Symptoms was used as a self-rated measure of depressive symptoms(QIDS-S; Bernstein et al., 2010). The Young Mania Rating Scale (YMRS; Young et al., 1978) was used to exclude current hypomania/mania, while historical self-reported hypomanic symptoms were assessed using the 33-item hypomania checklist (HCL-33; Feng et al., 2016). Melancholic symptoms of depression were assessed using the Newcastle Depression Diagnostic scale (NDDS)(Carney et al., 1965), and atypical depression with the Atypical Depression Diagnosis Scale (ADDS; Stewart et al., 1993). This latter scale provides four categories: (1) 'non-reactive depression', (2) 'simple reactive depression', (3) 'probable atypical depression' and (4) 'a definite atypical depression'. The presence of rumination and irritability symptoms was established by using the Rumination Scale (Treynor et al., 2003) and the Self-Assessment of Irritability Scale(Snaith & Constantopoulos, 1978). Anxious symptoms were assessed using the "Anxiety Factor" on the 17-item (HAMD-17; Levitt et al., 1993). Environmental factors in the three months prior to study participation were assessed using the Hassles Scale(Kanner & Coyne, 1981) and the Recent Life Changes Questionnaire(RLCQ; Miller & Rahe, 1997), while early life trauma was assessed with the Childhood Trauma Questionnaire (CTQ; D. Bernstein & Fink, 1994). The presence of childhood trauma was recorded if a participant presented with a score greater than the threshold on any of the following subscales of the CTQ: emotional abuse (threshold >12), physical abuse (threshold >9), sexual abuse (threshold >7), emotional neglect (threshold >14) and physical neglect (threshold >9). Patients were medication free for ≥2 weeks (≥4 weeks for fluoxetine) and were not receiving any psychological intervention at the time of the assessment. Healthy controls were free from current or past psychiatric diagnoses as assessed using the MINI, as were their first-degree relatives using patient history. Participants were excluded if they reported any illicit substance use in the previous three months or had any unstable medical condition. Healthy controls were free from current or past psychiatric diagnoses as assessed using the MINI, and reported no history of psychiatric illness in first degree relatives. Participants were excluded if they reported any illicit substance use in the previous three months or had any unstable medical condition. Controls were recruited from public advertisements and from hospital and university staff across the three sites from UK (n=15),

Chile (n=2) and Hong Kong (n=28). Controls were selected in order to match as closely as possible in age and gender with the depressed group. The local ethics committee approved the research and written informed consent was obtained from each participant. All participants received modest compensation for taking part in the research.

Fingernail specimens

Participants were instructed to clip their fingernails 15 days prior to the study assessment day in order to standardise the number of days' growth to be sampled (approximately 1.5 mm per nail). Subjects were provided with detailed instruction on how to cut their fingernails accurately at the desired length at the 15-day time point, store the samples correctly and post them in specific containers back to the investigators. Cortisol was subsequently extracted according to the method described by Warnock and others (2010), with minor modifications (please see supplementary material for details on the extraction protocol).

Statistical analysis

Data were checked for normality using graphic methods, such as histograms, and the Kolmogorov-Smirnov statistical test; results indicated that the fingernail cortisol data were not normally distributed (Figure 1). Therefore, we used a non-parametric test (Mann-Whitney U test) to compare fingernail concentration between MDE participants and healthy controls and the Kruskal–Wallis –test when cortisol values were compared in more than two groups. Demographic and clinical data were compared using parametric statistical tests (*t* tests) for continuous variables and *Chi*-squared for categorical variables. Finally, a regression model was created to estimate the regression coefficient (β) and 95% confidence intervals (CI) of the variance in cortisol levels in fingernails in relation to clinical and demographic continuous and categorical predictors in MDE participants. We used Generalised Linear Models (GLMs) with a gamma distribution and a log-link function to model the data so as to better take into account the right-skew in the hormone concentrations in the tissue. The GLM is a flexible generalization of ordinary linear regression that allows for response variables that have error distribution models other than a normal distribution (Nelder & Baker, 1972). The level of significance was set at $p \leq 0.05$ (two-tailed).

3. Results

Detailed demographic and clinical variables of the sample are presented in Table 1. Twenty-six subjects with a MDE including one patient with bipolar disorder type I and two with bipolar disorder type II took part in the research and were sex and age matched with 45 healthy controls. Twenty depressed patients were recruited in London (UK), six in Santiago (Chile) and none in Hong Kong. None of the patients showed significant current hypomanic symptoms. Eleven subjects met criteria for atypical depression (42%) whereas 15 were considered non-atypical (59%), based on the ADDS scale. As predicted, patients differed from controls on a number of clinical parameters including symptoms of depression and anxiety, ruminative thinking style, and early life and current environmental factors. There was no difference in the length of fingernail samples, waist circumference, or Body Mass Index (BMI) between groups.

Fingernail cortisol level in depressed subjects vs. healthy controls

Depressed subjects had significantly higher fingernail cortisol concentrations (FCC) (mean [SD]=201.2 [277.3] pg/mg, median [interquartile range, IQR]=96.4 [60.2–396.8 pg/mg]) in comparison to controls subjects using the Mann-Whitney U-test (mean [SD]=101.5 [90.5], median [IQR]=76.9 [39.2–165.6 pg/mg]; p=0.03) (see Figure 2). However, when we ran a sensitivity analysis excluding the 3 patients with bipolar disorder the difference between MDE and controls decreased to a trend level (p=0.09). There was no significant difference in FCC between different sites. FCC values for UK depressed participants were: mean [SD]=176.4 [235.9] pg/mg, median [IQR]=94.1 [70.1–154.8 pg/mg] and for Chilean depressed subjects were: mean [SD]=193.6 [268.3], median [IQR]=96.4 [77.5–160.8 pg/mg]; p=0.27 using the Mann-Whitney U-test. FCC values for control subjects were: UK: mean [SD]=142.6 [194.8] pg/mg, median [IQR] =88.8 [62.9–121.3 pg/mg], Chile: mean [SD]=100.0 [85.3], median [IQR]=84.6 [62.0–105.3 pg/mg]; and Hong-Kong: mean [SD]=101.5 [90.5], median [IQR]=76.9 [62.0–105.4 pg/mg]; p=0.29 using the Kruskal–Wallis -test.

Regression analyses of fingernail cortisol levels with clinical variables

The generalised linear model showed that higher levels of cortisol were associated with higher depression severity scores, a diagnosis of non-reactive depression, and more prominent melancholic symptoms (all $p<0.05$; Table 2). Conversely, lower levels of cortisol were associated with more severe fatigue ($p<0.05$; Table 2). No other clinical variables exerted a significant effect on cortisol levels in comparison to healthy participants (all $p > 0.05$).

4. Discussion

In this study we set out to measure differences in cortisol levels in MDE by using a novel technique utilising fingernails, representing cortisol concentrations averaged over a period of 15 days. We demonstrated that fingernail cortisol concentration is increased in MDE compared with healthy controls. The GLM also showed that higher levels of cortisol were associated with higher depression severity scores, features of non-atypical depression, and more prominent melancholic symptoms. Conversely, more severe fatigue was associated with decreased cortisol levels.

Medium-term cortisol levels

These results suggest that overall cortisol levels are elevated during an MDE when assessed cumulatively over a 15-day period, This provides additional evidence over and above findings of elevated shorter term measures of cortisol such as blood, saliva and urine, and strengthens the position of elevated cortisol release as an important neurobiological correlate of depression(Pariante, 2009). Further support for the elevation of cortisol over longer time frames comes from studies using hair specimens from depressed patients, which usually cover longer periods of around 3 months(Dettenborn et al., 2012; Herane Vives et al., 2015; Wei et al., 2015;Pochigaeva et al., 2017).

Clarification that raised cortisol is a persistent phenomenon suggests that depression manifests the same neurobiological disturbance as Cushing's syndrome, but in a less severe form. Chronic hypercortisolism in Cushing syndrome has a variety of potentially deleterious effects, including obesity, hypertension, glucose intolerance, osteoporosis, impaired immune function, and poor wound healing(Pennacchietti, 2015). However, the degree of hypercortisolism present in depression is of a milder form compared to that Cushing's syndrome. For example, one study found a median fingernail cortisol of 679 pg/mg in

Cushing's syndrome(Thomson et al., 2010), which is much higher than the median of 96.4 pg/mg in the current study, notwithstanding that direct comparison is hampered by some differences in the techniques used. Nevertheless, several depressed individuals did have fingernail cortisol levels in the 200-1000 pg/mg range (see Fig 1) which are more similar to those seen in Cushing's syndrome. Overall, however, the level of cortisol measured in depressed subjects appears to fall within the range described as subclinical hypercortisolism(Dalmazi & Pasquali, 2015). This condition has to date been generally been applied to subjects with 'adrenal incidentalomas' and not from depression. Given the potential impact of chronically elevated cortisol levels as demonstrated in fingernails, depression ought perhaps to be included within the remit of subclinical hypercortisolism.

The occurrence of depressive recurrences would be expected to lead to repeated, periodical high level of cortisol (Mueller & Leon, 1999). This could potentially contribute to factors such as poorly controlled blood pressure(Bednarek & Jankowski, 2014), raised glucose levels (Gillmer et al., 1975) and other more complex cardiovascular or metabolic condition often coexisting in subjects with subclinical hypercortisolism(Dalmazi & Pasquali, 2015) and depression (Katon, 2003). Another possible correlate of elevated cortisol is that of persistent cognitive impairment, which is present in some depressed patients and has been found to affect most of the cognitive domains (Porter et al., 2003). If the impaired cognition in patients with histories of more severe depression is linked to hypercortisolism, this raises the possibility that drugs such as mifepristone, a specific glucocorticoid receptor blocker, may have a therapeutic benefit in this neurocognitive impairment(A. H. Young et al., 2004).

Relation to depression sub-type

We found that higher fingernail cortisol was associated with more severe depression, and with depressive episodes characterised by melancholic and non-reactive mood of depression. This supports previous research suggesting that Hypothalamic–Pituitary–Adrenal (HPA) axis hyperactivity is more prominent in melancholic (Carroll & Feinberg, 1981) and psychotic (Schatzberg et al., 2002) sub-types of depression. Therefore, elevated cortisol levels may be a potentially relevant biomarker particularly for those particular forms of depression.

On the other hand, we did not find that fingernail cortisol was specifically linked to sub-type of atypical depression as the degree of broadly defined by the ADDS atypical symptomatology. Atypical depression has elsewhere been described to have lowered rather

than elevated cortisol levels (P. W. Gold & Chrousos, 2002). These previous studies assessed only short term cortisol levels (P. Gold & Chrousos, 1999; Lamers et al., 2013), and it is possible that hypocortisolaemia is not a chronic phenomenon in atypical depression.

However, we did find that one of the features typically associated with atypical depression, severe fatigue, was negatively correlated with cortisol levels. Our results, therefore, provide new evidence that indicates that this somatic symptom is linked to lowered cortisol levels, not only in other disorders which have commonly been associated with low cortisol levels, such as Chronic Fatigue Syndrome (A. J. Cleare, 2003), but also in affective disorders, such as MDE. It has been suggested that lowered cortisol may be an aetiological factor in CFS(A. Cleare, 2004), given that low dose cortisol replacement can alleviate the experience of fatigue (A. Cleare et al., 1999), which raises similar possibilities in patients with fatigue within affective disorders (A. Cleare, 2009).

Methods for assessing longer-term cortisol levels

Cortisol measurement from fingernails presents a number of advantages over hair extraction, the other novel specimen recently introduced for measuring chronic cortisol levels over longer periods. Specific confounding factors such as cosmetic treatment and frequency of washing for hair samples have not been found in fingernails, rendering results less susceptible to these extraneous factors, and facilitation easier matching of subject characteristics in case-control studies. Frequently, male subjects cannot be included in research using hair specimens because they are more likely to have a hair length shorter than three centimetres, the standard measure that these studies use (Dettenborn et al., 2010). Moreover, there remains a lack of agreement as to the optimal way to analyse hair samples (Pragst & Balikova, 2006). Whilst some studies have ground hair before extracting cortisol, others have only cut them in small pieces (Davenport et al., 2006). The procedure of hair sampling can also have an aesthetic effect, leading potential subjects to avoid providing hair samples (Izawa et al., 2015). In a recent community study, we found that 42% of subjects were unable to give hair due to shortness or baldness, while another 29% refused to give hair for various reasons (Fischer et al., 2016).

Limitations

There are some methodological issues in this study worthy of discussion. In terms of interpretation, measuring raw cortisol levels alone may not be the optimal way to assess its overall functional levels. Thus, it has been suggested that the ratio of cortisol to the adrenal androgen dehydroepiandrosterone (DHEA) may be a better index of functional cortisol levels(Goodyer, 1998). Future work could also measure DHEA in fingernails in depressed patients in order to obtain this ratio over longer time frames.

Some limitations in the use of fingernails include possible effects on cortisol levels induced by nail growth rate, which can vary with seasonal changes, sex, different finger digits, age, clipping frequency, nail filing, and nail-biting habits (Gupta et al., 2005), although common cosmetic products such as nail varnish use are unlikely to affect fingernail cortisol (Ben Khelil et al., 2011). Berker and others (2007) suggested that the length of fingernails is very important in relation to the concentration of cortisol extracted over a specific period of time. However, there were no differences in the length of fingernails between cases and controls in our sample, which indicates that the period covered by the nail specimens in this study was comparable between cases and controls.

Future, larger studies using fingernails could compare more directly the degree of hypercortisolemia that depressed subjects present in comparison to subjects with clearly established hypercortisolism, such as those with Cushing's syndrome, and link this to the presence of adverse metabolic and other physical effects. Studies using fingernails could also investigate cortisol levels in other specific non-atypical subtypes of depression, such as those with DSM specifiers such as anxious-distress, melancholic or psychotic features. Future studies might also investigate whether chronic hypercortisolemia in fingernails is a specific finding of unipolar or bipolar depression; given the small number of MDE patients with longitudinal diagnoses of bipolar disorder in the current study we have been unable to do this. This analysis seems particularly important since the exclusion of the small number of patients with bipolar disorder decreased the difference in cortisol levels compared to controls from a significant to a trend levels. Longitudinal studies may also be helpful in validating the pattern of cortisol secretion in relation to other features of depressive illness such as remission, relapse, chronicity and comorbidity. Prospective cohort studies in young people with depression could evaluate whether cortisol may play a causative role in the association between medical conditions, such as diabetes and heart disease, and depression.

5. Conclusions

The use of fingernails for measuring cortisol levels aggregated over 15 days showed that depressed subjects have elevated medium-term cortisol levels in comparison to control subjects. Demonstrating the longer term nature of the elevated cortisol suggests that depressive episodes may be part of a group of conditions that have subclinical hypercortisolaemia. The role of cortisol in medical conditions that occur at higher rates in depressed subjects need to be investigated. The present results also suggest that patients with more severe depression, and with non-reactive and melancholic features, are particularly at risk of elevated cortisol.

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Supplementary material

Fingernails extraction

Fingernail samples were washed two times with 3 ml isopropanol (LC/MS grade) in glass vials and dried overnight. Next, 20-50 mg of the washed clippings were ground (Retsch ball mill mixer; 30 Hz) and 10-25 mg of accurately weighed specimen was used for cortisol extraction (1.5 ml LC/MS methanol; 1 hour on rotary mixer). Finally, after centrifugation, 1.3 ml of the methanol supernatant was transferred to a separate tube and evaporated to dryness

at 60-degree Celsius under nitrogen. The residue was redissolved in 1 ml of assay buffer and stored at -30 degree Celsius until Immunoassay(Mondelli et al., 2010). All fingernail samples were analysed at the Bethlem Royal Hospital, London UK.