Elevated fingernail cortisol levels in major depressive episodes

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ABSTRACT

Background: The extent to which cortisol levels are elevated in major depressive episodes (MDE), and hence could act as a biomarker of illness, remains unclear. Although patient characteristics may explain some of this variation – for example elevated cortisol being more often found in patients with severe, psychotic or melancholic depression – problems with the methods used to measure cortisol may also have contributed to the inconsistent findings. Fingernails are a novel sample that can be used to assess aggregate cortisol concentrations over a 15-day period, and may provide a more accurate reflection of longer term cortisol level changes in MDE and help clarify this issue. This methodology has not yet been utilised in MDE.
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 considered a candidate biomarker potentially able to support the diagnosis or sub-typing of the major depressive disorder at biological level (Pariante 2009). However, there is some inconsistency in this finding, especially when using short-term measurements of cortisol concentrations such as salivary cortisol. This may be, in part, explained by the large heterogeneity seen within depressive syndromes. Indeed, it has been shown that depressive episodes with psychotic or melancholic features (Carroll et al., 2007; Carroll et al., 2012; Schatzberg et al., 2002), largely account for the increased cortisol levels found in this disorder. Furthermore, this could be also 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 and Wüst, 2010). Fingernails, an integumentary type of tissue that share several properties with hair (Herane Vives et al., 2015), 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 of time. 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.

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2. Methods

2.1. Participants

Participants were recruited as part of an ongoing collaboration between 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. Twenty-six depressed patients were recruited, twenty in London (UK), six in Santiago (Chile) and none in Hong Kong. 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 and Lecrubier, 1997) 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 ($\geq$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 (Rush et al., 2003). The Young Mania Rating Scale (Young et al., 2004) 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 Diagnostic 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 and 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 et al., 1981) and the Recent Life Changes Questionnaire, while early life trauma was assessed with the Childhood Trauma Questionnaire (CTQ; Bernstein and 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 $\geq$2 weeks ($\geq$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. Controls (n = 45) 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). The over recruitment of controls from Hong-Kong was undertaken in order to increase the statistical power of the study. 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.

2.2. 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.

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

2.4. 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 (Fig. 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 and 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.

3.1. 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