KING´S COLLEGE LONDON

Institute of Psychiatry

Mphil Psychological Medicine

Protocol Study:

Assessment of hypothalamic–pituitary–adrenal axis in atypical and non-atypical major depressive disorder, bipolar depression and chronic fatigue syndrome.

(HPA HAIR STUDY)

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February 14st 2012.

1.-Background:

Key words: hair cortisol concentration (HCC), hypothalamic–pituitary–adrenal (HPA), Chronic fatigue syndrome (CFS), Mayor depressive disorder (MDD), Mayor depressive disorder non atypical (MDD-NA), Mayor depressive disorder atypical (MDD-A) Bipolar depression (BD), area under curve (AUC*), Body Mass Index (BMI) Hamilton depression scale for anxiety,* (*HAM-A), cortisol awakening response (CAR), centimetre (cm). Mini-International Neuropsychiatric Interview (MINI) Atypical Depression Diagnostic Scale (ADDS),Chalder Fatigue Scale (CFS), Childhood Trauma Questionnaire (CTQ) treatment resistant bipolar depression (TRBD), treatment resistant unipolar depression (TRUD), Experience Sampling Method (ESM), International Affective Picture System (IAPS), Montgomery–Åsberg Depression Rating Scale (MADRAS-Cl), the Temperament Evaluation of the Memphis, Pisa, Paris, and San Diego Auto questionnaire (TEMPS-A), Young mania rating scale (YMRS), Pittsburgh Sleep quality sleep (PSQS), Barratt Impulsiveness Scale version 11 (BIS-11),Harkavy Asnis Suicide Survey-II (HASS-II). Positive Affect and Negative Affect Schedule (PANAS), recent life changes questionnaire (RLCQ), Hipomanic symptoms check-list (HCL-32), Zung Self-Rating Anxiety Scale (ZAS)*

The HPA axis consists of a chain of stimulatory hormones and feedback loops and is under control from the higher cerebral centres that determines its overall activity (Papadopoulos and Cleare, 2012). One of these hormones is cortisol which regulates a wide range of bodily functions including metabolism, immunity, neuronal survival, neurogenesis (Dettenborn et al., 2011,) memory retrieval (Buchanan and Tranel, 2008) processing of emotion (Skosnik, et al. 2000) *and stress response* (*Chrousos, 2009)*

There are also several psychiatric illnesses related to an abnormal response to chronic stress and therefore in cortisol concentration, but with some controversy about the directionality and intensity of that response.

Mostly, that controversy arises from the different types of specimens used to assess cortisol concentration because there are many different matrixes to assess levels of cortisol such as, blood, urine, and saliva and all of them have some advantages and disadvantages.

For example, saliva sample provides a non invasive procedure which reflects the biologically active fraction of cortisol due to only unbound cortisol can enter saliva. This specimen has also a high correlation with blood cortisol concentrations *(Vining et al, 1983*) *and allows to assess different indicators of HPA activity such as: daytime cortisol profile and event-related designs. Until now, it has been developed several different ways to assess cortisol output such as Δ between waking and bedtime cortisol levels, the diurnal slope, total output as*

measured by the AUC (Adam et al., 2009) and the cortisol surge that takes place during the first hour after awakening, the so-called (CAR), which represents a response to morning awakening and is distinct from the circadian rise in HPA axis activity in the morning hours. However, at the same time all of these measures have yielded several controversies in terms of their results.

Related to that, the majority of previous reports have shown elevated saliva cortisol secretion in MDD than in controls (Vreeburg et al., 2009) but those studies refer to cortisol concentrations in one single point assessment. Previous reports say also that other types of MDD such as atypical depression would have hypocortisolism (Gold and Chrousos, 2002) as others psychosomatic chronic stress-related diseases, such as CFS (Papadopoulos and Cleare, 2012) but there is no convincing evidence that overall HPA activity in that group. (O' Keane et al, 2012). BD has also been involved in these controversies, in a recent study Markopoulou et al, 2012 has found a heightened CAR -as measured by the area under the curve- in TRUD and an opposite pattern, a reduced secretion of cortisol following awaking, in patients with TRBD, and both groups in comparison with healthy controls. While Kamali et al. (2012) did not find any difference in that group of patients.

For that reason saliva specimen is just useful to obtain a picture of the level of cortisol at real time; in other words, a good measure of acute concentration, but they are not adequate matrix to obtain a measure of chronic cortisol levels.

Therefore, there is a critical need for the establishment of a biomarker that accurately measures intensity and course over time of cortisol (Kudielka et al. 2012) *under daily life circumstances due to the association between an abnormal chronic cortisol production and mental health problems.*

In that sense, recently, hair as a new specimen has been introduced and validated as a new specimen able to solve that problem measuring chronic cortisol concentration.

In relation to neurobehavioural tasks and cortisol, Abercrombie et al., (2003) showed that healthy subjects viewing negative pictures did not cause endogenous cortisol elevations using IAPS task. But that research has not been studied in affective or CFS disorders to assess cortisol reactivity to stress. A second task, the processing of facial emotion expression has revealed that there is reasonably consistent evidence of a negative response bias towards sadness in individuals with major depression, so that positive (happy), neutral or ambiguous facial expressions tend to be evaluated as more sad or less happy compared with healthy control groups (Bourk et al., 2010). Recently, one study examined the relation between facial emotion processing accuracy (Facial Expression Recognition Task) and an aspect of hypothalamic–pituitary–adrenal axis function (Dexamethasone Suppression Test) in 64 inpatients with major depression and 49 healthy controls over a 2-week period at baseline and 10–14 days after baseline. The results showed an increased cortisol response to dexamethasone in blood samples was significantly correlated with reduced ability to recognize facial expressions of anger, sadness and disgust within the total sample, but these correlations did not remain significant at 10–14 days. However and surprisingly, in that sample the cortisol response to dexamethasone was comparable in acutely depressed inpatients and healthy controls, and did not change over time in relation to treatment response. This task had not been also studied in different types of depression or CFS using hair cortisol and different measures of saliva cortisol.

There are also two controversial term in mood disorders which have not been studied in relation to long term cortisol levels. One of them is affective temperaments (emotional reactivity) which embrace both affective liabilities and assets (Akiskal H. and Akiska K., 1988, 1992; Akiskal, 1996). These affective temperaments would be cyclothymic, dystimic, irritable, hyperthymic, and anxious (Akiskal et al, 2005). They have been considering as dispositions to mood states; conceptualizing them as "subaffective" or trait affective expressions of mood

disorders (Akiskal, 1981). Moreover, it has been hypothesized to precede and follow episodes of mood disorders in an affective trait-affective disorder continuum (Akiskal et al., 2005).

The second one is ruminations; this refers to cognitions that repeatedly focus the depressed individuals' attention on their depressed mood as well as the potential causes and consequences of their depressed mood. (Nolen- Hoeksema, 1991, Lyubomirsky, Caldwell, & Nolen-Hoeksema, 1998; Nolen- Hoeksema, 1991).

The Response Style Theory (RST) (Bagby R. and Parker D., 2001) predicts that ruminating about one's depression prolongs and exacerbates depression. This interpretation is also consistent with the notion that rumination, in particular, self-focused rumination is a dispositional, trait-like construct, as this type of rumination could occur in the absence of depressed mood; symptom rumination, by definition, could not occur in the absence of symptoms on which to focus.

In this study, for the first time, hair cortisol specimen will be using, in addition to saliva measures (CAR, AUC and cortisol reactivity) to assess chronic cortisol levels, clinic (symptomatic and temperamental) and neurobehavioural features in four different psychiatric illnesses (MDD-NA, MDD-A, CFS and BD) which there have arisen several controversies about the role of cortisol in the neurobiology of these illnesses, opening the possible to find in this specimen in combination with others tools a real and truly biomarker for psychiatric diagnosis and monitoring treatment outcome.

2.-Aims and Objectives:

This research is focused on investigating cortisol levels in relation to three clinical subtypes of depression and in patients with CFS. To date, studies have only been able to obtain cross sectional *"snapshots"* of cortisol status. The novel methodology of hair cortisol analysis allows the assessment of cortisol levels over periods of weeks and months. The

main purpose of this study will be to use cortisol analysis of between 25 to 100 mg of hair in order to gain an improved measure of long-term HPA axis function in these patients and in a group of healthy controls. The study will use a case-control study design and measure hair cortisol in four groups of patients: 1) MDD with atypical features; 2) MDD with non-atypical features; 3) bipolar depression; and 4) CFS. In addition, measures of salivary cortisol will be taken to cross-validate the hair cortisol measures. Hair and salivary cortisol will be reassessed after a period of treatment.

3.- Hypotheses:

First hypothesis: – Atypical MDD shows long term hypocortisolaemia in hair specimen in comparison with a control group. Atypical MDD shows hypocortisolaemia in saliva specimen through AUC, as a measure of long term cortisol secretion in comparison with a control group. Atypical MDD shows hypercortisolaemia in saliva specimen through CAR, as a measure of short term cortisol secretion in comparison with control group. Atypical MDD shows hypercortisolaemia in saliva specimen post IAPS task , as a measure of cortisol reactivity in comparison with control group.

Second hypothesis: – Non-atypical MD shows long term hypercortisolaemia in hair specimen in comparison with a control group. Non-atypical MDD shows hypercortisolaemia in saliva specimen through AUC, as a measure of long term cortisol secretion in comparison with a control group. Non-atypical MDD shows hypercortisolaemia in saliva specimen through CAR, as a measure of short term cortisol secretion in comparison with control group. Non-atypical MDD shows hypercortisolaemia in saliva specimen post IAPS task, as a measure of cortisol reactivity in comparison with control group.

Third hypothesis: – Bipolar depression shows long term hypocortisolaemia in hair specimen in comparison with a control group. BD II shows hypocortisolaemia in saliva specimen through AUC, as a measure of long term cortisol secretion in comparison with a control group. BD II shows hypocortisolaemia in saliva specimen through CAR, as a measure of short term cortisol secretion in comparison with control group. Bipolar depression shows hypercortisolaemia in saliva specimen post IAPS task, as a measure of cortisol reactivity in comparison with control group.

Fourth hypothesis: – CFS shows long term hypocortisolaemia in hair specimen in comparison with a control group. CFS shows hypocortisolaemia in saliva specimen through AUC, as a measure of long term cortisol secretion in comparison with a control group. CFS shows hypocortisolaemia in saliva specimen through CAR, as a measure of short term cortisol secretion in comparison with control group. CFS shows hypercortisolaemia in saliva specimen post IAPS task, as a measure of cortisol reactivity in comparison with control group.

Fifth hypothesis: - Hair cortisol levels reflect changes in HPA axis function with treatment, as also assessed simultaneously by salivary cortisol measures.

Sixth Hypothesis:- Long-term hypocortisolemics disorders in hair specimen, show reduce memory retrieval and storage through IAPS task in comparison with control group. Long-term hypercortisolemics disorders show reduce memory retrieval and storage through IAPS task in comparison with control group.

Seventh hypothesis:– Long-term hypercortisolemics disorders show reducing ability to recognize facial expressions of anger, sadness and disgust through facial emotion processing task in comparison with control group. Long-term hypocortisolemics disorders reducing ability to recognize facial expressions of anger, sadness and disgust through facial emotion processing task in comparison with control group.

Eighth Hypothesis: Affective temperaments in control group show long term normocortisolaemia in hair specimen in comparison with affective temperaments in MDD-NA, MDD-A, BD and CFS groups

Ninety hypothesis: Subjects with ruminations in control group show long term normocortisolaemia in hair specimen in comparison with subjects with ruminations in MDD-NA, MDD-A, BD and CFS groups

4. - Methodology:

4.1- Measures/ scales:

All subjects controls and cases will be administrated: MINI (Sheehan et al.,1998), CDC criteria (Fukuda et al, 1994), ADDS (Stewart et al., 1993), YMRS (Young et al.1978)., HCL-33 (Angst et al. 2005), RLCQ-modified (Miller and Rahe, 1997), CTQ (Bernstein *et al.,* 1994), Rumination scale (Treynor *et al*. 2003), as a basis diagnosis scales at the baseline.

It will be also administrated the following inventories for cases: HAM-A (Hamilton, 1959), CFS (Chalder *et al.,* 1993), Newcastle depression diagnostic scale (Carney *et al*. 1965), TEMPS-A (Akiskal *et al*. 2005), MADRAS-Cl (Montgomery *et al.*1979), PSQS (Buysse et al.1991), BIS-11 (Patton et al. 1995)., HASS-II, (Harkavy F. and Asnis GM, 1989), The hopelessness scale (Beck and Weissman 1974), A clinical scale for the self-assessment of irritability (Snaith et al.,1978), ZAS (Zung, 1971)

4.2-Biological specimens:

4.2.1-Hair cortisol analysis

Hair cortisol levels will be determined in all cases and controls. Hair samples will be collected at baseline, and after three months of treatment. The presence and frequency of any procedures which could affect the hair cortisol levels will be measured, including dye use and frequency of hair washing. The procedure for analysis will be as described in detail in Kirschbaum *et al.,* (2009). Briefly, hair cortisol analysis will be carried out in two stages. During the first stage samples of hair will be taken from the posterior vertex of the scalp in order to obtain between 25 to 100 mg of hair. As one of the main objectives of this research is to know the chronic cortisol levels pre and post-treatment, the hair length (cm) will depend on when the treatment with psychotherapy or psycho tropics have started. It means that we can recruit subjects that they have been receiving treatment until previous four months, because HCC is universally agreed to be reliable up to at least 5cm from the scalp (Russell et al, 2012). Depending, how long the subject's hair are. In this case, it will be analyzed between 25 to 100 mg of hair of the 5th farthest cm to the scalp in the first hair sample. In the second hair sample, it will be analyzed between 25 to 100 mg of hair of the first closest cm to the scalp in order to obtain the real effect of the treatment in chronic cortisol levels. If we analyzed the levels of cortisol of the second and third cm during the treatment we would obtained the HCC during the treatment on average and non post treatment effect. The hair length will be also adjusted by ethnicity because African, Asian, and Caucasian individuals have different hair growth rates (288 ± 51, 421 ± 53, 371 ± 59 μm/day, respectively) (Loussouarn et al., 2005). Then, the sample of hair will be washed with isopropanol and then pulverised in a mixer mill. An accurately weighed quantity of the powder hair will be treated with methanol to extract cortisol which will be kept at – 40 c. During the second stage the extracted cortisol will be quantified with an automated

established chemiluminescence assay (CLIA) used for saliva cortisol estimations. Two samples will be taking at the baseline and at the end of the treatment.

Hypercortisolaemia in hair cortisol sample is defined as levels higher than 26.7 pg/mg in the first 3 cm of hair to the scalp and 21.9 pg/mg in the second 3 cm of hair to scalp based on the study of Dettenborn et al. (2011). Hypocortisolaemia in hair cortisol sample is defined as levels lower than 11.802 pg/mg in the first 3 cm of hair to the scalp and 8.894 pg/mg in the second 3 cm of hair to scalp based on the study of Steudte and Dettenborn et al. (2010).

4.2.2-Salivary cortisol analysis:

Samples will be taken for both the diurnal cortisol profile (CAR and AUC) and cortisol reactivity.

4.2.2.1- Diurnal cortisol profile (CAR and AUC):

The samples will be taking at patient's residence on the same weekday except Mondays, once every month for three months for all participants. Samples will be taken at 0, 30 and 60 minutes after awaking, 1200h, 1600h and 2000h., according to Roberts´s protocol for saliva collection (Robert *et al.,* 2004). These specimens will be collected at home, storage in the fridge and send them back by post to the laboratory of Bethlem Royal Hospital. Control's subjects will also have to fill out a structured diary technique to assess mood and anxiety in the context of their daily living environment: ESM. All self assessment will be rated on *5 point likert scale* asking for the anxiety, stress and mood level for every saliva measure. Saliva specimen will be collected in *salivettes* using cotton wool swab method.

4.2.2.2-Cortisol Reactivity:

Previous report has shown that cortisol levels were highest 10 min after cessation of stress (Kudiela et al. 1998). Cortisol reactivity will define as an increase in cortisol levels

in response to the IAPS task (see below). Then, two cortisol saliva samples will be collected, before and 10 min after the task ends as a measure of cortisol reactivity.

4.2.3- Neuro behavioural tasks (total time of IAPS and Facial Emotion Processing Task: 25.9 min)

All participants will be invited into the lab for one session:

Participants will be instructed to eat a light dinner at least 1 hr prior to the initial session and to refrain from eating, exercising, and drinking anything but water for the hour prior to session. Participants will be also instructed to refrain from drinking alcohol for the 24 hr prior to both sessions. Individuals who are occasional smokers (i.e., less 1 pack per month) were instructed not to smoke for the week prior to the sessions. Participants will be tested individually, and tasks will be administered on a computer, with the exception of self-report questionnaires and the free-recall tasks.

4.2.3.1-IAPS (17.9 min)

Measurement of emotional state: To examine the relation between cortisol levels and subjective emotional experience, current emotional state will be measured two times: immediately prior and post to task administration Ratings were obtained on the 20 adjectives from the PANAS (Watson et al., 1988)

Encoding of negative stimuli:

Rating task; (4.6 min)

All participants will perform a picture-rating task, which exposed them to negative stimuli. Pictures (i.e., photographs) will be chosen from the International Affective Picture System (IAPS; Lang et al., 1998). For both the picture one sets of stimuli (deemed Sets A and B) will be developed to allow for counterbalancing of targets and distracters in later tests of recognition memory. Because counterbalancing adds another factor to the study design and introduces variability, we will choose to limit counterbalancing of variables to the

recognition memory task. Recognition memory targets and distracters will be considered the most important items to counterbalance in order to ensure that memory effects will be not related merely to a particular set of stimuli. Thus, other variables, such as order of presentation of the rating tasks, will be kept constant for all participants.

Both picture sets included 28 negative pictures that will be matched on average normative ratings of arousal (Lang et al., 1998). To facilitate free-recall testing, content overlap among pictures will be minimized within each set.

During the rating tasks, participants will be instructed to rate pictures on the basis of how they feel while viewing each stimulus, and will be not told that they would later be asked to recall the stimuli. Participants will rate pictures (5-s stimulus presentation duration) on one 9-point numeric *likert scales* assessing valence per set of pictures.

Memory assessment:

Explicit memory will be assessed for the stimuli in the picture-rating tasks. Participants will be not given feedback on their performance for any of the memory tests.

Free-recall: (11 min)

Participants will complete separate free-recall tasks for pictures, in which they will be instructed to list briefly describe all the pictures they can remember from the rating tasks. Participants will be given 11 min to complete the picture free-recall tasks. In addition to number of correct responses, free-recall tasks will be also scored for intrusive errors, that is, errors of commission, which will be responses that will be not presented in the descriptions of pictures that will be not presented in the picture rating task. All pictures free-recall lists will be scored by one person because scoring the free-recall lists for pictures entail a degree of subjectivity.

Recognition memory: (2.3 min)

After the free-recall tasks will be completed, separate recognition memory tests for pictures will be administered. The tasks will involve use of a "yes" or "no" questions to indicate whether or not test stimuli will be presented during the rating tasks. Half of the test stimuli will be targets (previously viewed), and half will be distracters (new stimuli). Instructions emphasize both speed and accuracy. Distracters will be pictures from the alternate set of stimuli not presented during encoding (i.e., A or B, accordingly). Only half of the stimuli from each Set A and B will be used for the session recognition memory tasks. The subsets of stimuli chosen for the recognition tests from Sets A and B will be psychometrically matched on normative ratings.

The participant's ability to discriminate between previously presented and new items, (i.e., "sensitivity") served as the dependent variable for recognition memory. The sensitivity index Pr will be used (Snodgrass & Corwin, 1988). Pr is the proportion of old items (targets) endorsed minus the proportion of new items (distracters) endorsed, that is, hits minus false alarms, with positive scores reflecting more hits than false alarms. This metric does not require that the data be normally distributed, and it provides a measure of sensitivity that is independent from bias (Snodgrass & Corwin, 1988).

Saliva sampling;

Salivary cortisol will be collected before and 10 minutes after finishing the task as a measure of cortisol reactivity.

4.2.3.2-: Facial Emotion Processing Task: (8 min)

Participants will be shown a series of photos of facial expression in a computer monitor and asked to decide which emotion (neutral, happy, angry, sad and fearful emotions) is shown in the faces by pressing the buttons. Each face will be presented until a participant

press the button and participants' responses will be measured for their reaction time and accuracy.

4.3-Sample:

4.3.1-Inclusion criteria:

Outpatients: DSM-IV criteria for relevant psychiatric disorder, i.e. (1) moderate to severe major depressive episode, (score of 17 or higher in HAM-D), single or recurrent, subtype with atypical features, non treatment resistant, (< 2 episodes not response or partial response to treatment) , psychotropics free at the period of time corresponding to the among of hair which will be analysed in the first hair sample, (MDD-A); 2) moderate to severe major depressive episode, (scores of 17 or higher in HAM-D), single or recurrent, ,without atypical features (MDD-NA), non treatment resistant (< 2 episodes not response or partial response to treatment), psychotropics free at the period of time corresponding to the among of hair which will be analysed in the first hair sample; 3) moderate to severe bipolar depressive episode (BD) (scores of 17 or higher in HAM-D), single or recurrent, in bipolar II disorder or patients with a previous history of an episode of hypomania, non treatment resistant (< 2 episodes not response or partial response to treatment) psychotropics free at the period of time corresponding to the among of hair which will be analysed in the first hair sample and 4) CFS will be defined using the consensus CDC criteria (Fukuda et al, 1994) psychotropics free at the period of time corresponding to the among of hair which will be analysed in the first hair sample . Any gender. Age range 18 to 65 years.

Controls: Absence of current or past history of psychiatric disorder. Age range 18-65 years. Any gender. Controls will be age and gender matched to patients.

4.3.2.-Exclusion Criteria: for all patients

1.-Medical problems potentially affecting the central nervous system or HPA axis , such as a current or past neurological disorder, head trauma, hypertension, myocardial infarction or ischemia, diabetes, hypothyroidism, Cushing's disease, or drug/ alcohol abuse.

2. –Dementia.

3. -Schizophrenia spectrum disorder.

4. -Personality disorder.

5. - Use of steroid medication.

6.- Hair length less than the among which would be possible to analyze in the first hair sample.

7.- Patients who have been using psychotropics which do not allow to measure chronic cortisol levels in the first hair sample, as a measure pre treatment.

8.- Patients who have been attending to any psychotherapy before the baseline. Which do not allow to measure chronic cortisol levels in the first hair sample, as a measure pre treatment

9.- Background of anaphylactic reactions to antidepressants.

10.- Any subjects with high suicidal risk.

11.- Hypersensibility to any medications.

12.- Sleepiness secondary to any other medical condition E.G: narcolepsy, morbid obesity.

13.- Any dependence to drugs or medication unless nicotine.

14.- IQ less than 85 points according north america ability reading test scale (NAART)

www.brainmetric.com/pdfs/naart.pdf

4.3.3-Power Calculation:

The first power calculation is based on the expected degree of hypercortisolaemia in the group of patients with non-atypical MDD further to the findings in hair cortisol by Dettenborn *et al.,* (2011). The mean of depressed patients was 21.9 pg/mg, controls 13.4 pg/mg, with a composite standard deviation of 15.7 pg/mg. According to the calculator at http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html the expected sample size is n=40 per group (MDD-NA and controls) with a power of 80% and $p<0.05$. The second power calculation is based on the expected degree of hypocortisolaemia in the other three groups (atypical MDD, BD and CFS) based on the study of Steudte et al 2011. In this study there was a large effect size of 1.2, which gives a sample size of n=11 per group with a power of 80% and $p<0.05$. However, we anticipate that there may be a somewhat smaller effect size than this, and propose to recruit n=20 patients from each of these groups.

5.-Methods:

5.1-Recruitment: Day 0

Stage 1; Screening (15-30minutes): The study will be advertised to the general public and to outpatients within SLAM the Mood Anxiety and Personality Clinical Academic Group specialist services (MAP CAG). Interested potential participants will be contacted and a telephonic screening will be used as guidance for selecting potential suitable participants. Interested participants will be given a comprehensive description of the study and written information will be e-mailed or posted to them. In addition, the potential to augment recruitment through other clinics in Sao Paulo, Brazil, and in Santiago, Chile, will be explored during the initial phase of the PhD. Control subjects will be recruited from the

catchment area. Advertising, circulars and email will be using to recruit subjects. They must be mental and physical healthy and will meet all inclusion criteria..

Stage 2; Clinical interview (approximately 2 to 3 hours): Potentially suitable participants will be invited to attend a clinical interview within our clinical/research facilities. The researcher will offer opportunity to discuss any aspect of the study requiring clarification and answer any query which may arise. The researcher will seek informed consent for participation (see consent form). The research requires agreement for a letter of notification and comprehensive psychiatric assessment + treatments plans (for patients) to be sent to their general practitioner (a copy will also be made available to participants).

5.2- Basic diagnosis: day 1(All participants)

The actual plan at this stage will involve the following:

5.2.1.- Clinician administered rating scales:

In depth diagnostic clinical interview with experienced psychiatrist aided by diagnostic tools for DSM IV (MINI) and rating scales as applicable:
(HAMILTON/MADRS/ADDS/CDC/New castle /YMRS).

This stage aims to provide accurate diagnosis and precise recommendation for future treatment to the general practitioner according our evidence based treatment guideline and as used in our tertiary referral centre for affective disorders.

Subjects will screen by questionnaire, medical history and review of medical records. For demographic and health-related characteristics (gender, age, weight, BMI, waist circumference, smoking status, and current medication intake), participants asked to

answer a short questionnaire. Previous psychotropics status and dose age in milligrams will determine.

Subjects will be allocated in each group depends on the results of MINI, ADDS, HCL-33 and CDC: 1) Control group (orange) MINI (-) for any psychiatric disease, 2) MDD non atypical group (Blue): MINI (+) for MDD and 1 or 2 points in ADDS, 3) MDD atypical group (red)MINI (+) for MDD and 3 or 4 points in ADDS, 5) BD (green) MINI (+) for current mayor depressive episode and HCL-33 (+) as a diagnosis of for bipolar disorder II. 4) CFS (yellow): CDC (+) for CFS MINI (-) for any psychiatric disease.

5.2.2- Facial emotional processing task.

5.2.3- Hair cortisol (1)

5.2.4-Self-administered rating scales: Participants will be given self-administered rating scales to take away for completion including: RLCQ, CTQ , CFS ,Rumination scale, TEMPS-A, PSQS, BIS-11, HASS-II, The hopelessness scale, A clinical scale for the self-assessment of irritability HCL-33, RLCQ-modified, ZAS

5.3- Basal assessment: (days 2 and 3)

Day 2 (at home)

Cortisol diurnal saliva profile (AUC and CAR)

Day 3 (at laboratory)

IAPS and two cortisol saliva sample before and 10 min post-task. (1)

Delivering of Cortisol diurnal saliva profile (AUC and CAR) of day 2

5.4- Following up:

5.4.1.- Control group:

Every month: 1.- weight, BMI and waist circumference and weight.

2.-Cortisol diurnal saliva profile (AUC and CAR) and ESM.

5.4.2.- MDD-NA, MDD-A, BD and CFS groups:

Every month: 1.-Scales: MADRAS-CL, ZAS, PSQS and CFS

2.- Weight, BMI and waist circumference and weight.

3.-Cortisol diurnal saliva profile (AUC and CAR) and ESM.

5.5-End of Study: Day 90

5.5.1.- Control group:

1.- Scales: RLCQ, PSQS and rumination scale.

2.- BMI, waist circumference and weight.

3.- IAPS and two cortisol saliva samples before and 10 min post-task. (2)

4.- Facial Emotion Processing .

5.- Hair cortisol(2).

5.4.2.- MDD-NA, MDD-A, BD and CFS groups:

1.- Scales: MADRAS-CL, ZAS, HAM-A, efficacy index, RLCQ-modified, CFS and rumination scale, PSQS, BIS-11, HASS-II, The hopelessness scale, A clinical scale for the self-assessment of irritability , HCL-33.

2.- BMI, waist circumference and weight.

3.- Hair cortisol (2).

3.- IAPS and two cortisol saliva sample before and 10 min post-task. (2)

4.- Facial Emotion Processing .

5.4-Treatment:

Each group will receive a treatment for MDD-NA, MDD-A, BD and CFS based on NICE guidelines for each illness. Each patient will be reassessing every month. Treatment could be readjusted according tolerability, side effects and efficacy. The specific treatment is not an objective of this study.

6. - Statistical Analysis:

6.1-Statistical Analysis of Demographic Data:

We will examine clinical and socio-demographic differences between groups using one-way analysis of variance (ANOVA), F-test, or chi-square test. For post-hoc analyses we will use the least-significant difference (LSD) test.

6.2-Statistical Analysis of Clinical Data:

For the analysis between atypical MDD input variables (nominal) with hair cortisol analysis output variable (nominal: hyper/hypocortisolaemia), non-atypical MDD input variables (nominal) with hair cortisol analysis output variable (nominal: hyper/hypocortisolaemia), BD input variables (nominal) with hair cortisol analysis output variable (nominal: hyper/hypocortisolaemia), CFS input variables (nominal) with hair cortisol analysis output variable (nominal: hyper/hypocortisolaemia), t-test, medians, parametric and non-parametric statistics test will use.

For the comparison between average all data over 12 weeks of saliva diurnal cortisol profile: AUC and CAR input variables (quantitative normal) with hair cortisol analysis output variable (quantitative normal), we are going to use linear regression.

For the comparison between hair cortisol levels at baseline input variables (quantitative normal) with hair cortisol analysis at the end of treatment output variable (quantitative normal), we are going to measure the effect size of treatment.

Statistical analyses will be performed with the Statistical Package for the Social Sciences (SPSS 16.0).

8.-Ethical considerations:

Before starting the study, ethical approval will be sought and all participants will be asked to provide written informed consent prior to study participation. All study procedures will be in accordance with the Declaration of Helsinki.

9.-Relevance and importance:

Hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) is one of the major biological findings in depression, but the mechanisms underlying this abnormality are still unclear (Pariante, 2008). However, researchers have found that overall about 50 per cent of depressed patients show a picture of hypercortisolaemia (Schatzberg et al., 2002). However, this varies with the symptomatic picture; rates are higher in those with features of DSM-IV melancholic depression, strong somatic symptoms or psychosis (Schatzberg et al., 2002).

Until now it is quite unknowing the neurobiology of one sub-type of depression called atypical MDD, a well established subtype (Sullivan et al., 1998) present in between 15% to 30% of patients with MDD (Gold and Chrousos, 1999) with a relatively poor outcome when treated with standard antidepressants (Zubieta et al, 1999). In 1999, Gold and Chrousos suggested that atypical MD – which is characterized by mood reactivity and reversed vegetative symptoms (over-eating, over-sleeping, interpersonal rejection sensitivity) (DSM-IV-TR, 2000) – is associated with hypocortisolaemia rather than hypercortisolaemia (Gold & Chrousos, 1999). Juruena and Cleare have summarised work to date supporting this notion (Juruena and Cleare, 2007).

Also, it has been suggested that atypical MDD shares some features in common with CFS, which also has evidence for the presence of hypocortisolaemia (Juruena & Cleare, 2007; Papadopoulos and Cleare, 2012). Furthermore, atypical features of depression are more prevalent in depressive episodes experienced by bipolar patients (Benazzi, 2001) and interestingly recent research from this project's supervisors has shown that bipolar depression is also associated with hypocortisolaemia (Markopoulou et al., 2012).

There are, however, some inconsistencies in the above findings. For example, other studies have shown no significant difference in saliva cortisol levels between non-atypical and typical MD using the CAR (Vreeburg et al., 2009). In a recent review, it was suggested that the premise that atypical depression is associated with hypofunction of the HPA axis is in fact not well supported by the literature, with insufficient evidence as yet that those with atypical MD have low cortisol levels (O´ Keane et al., 2012). Indeed, the main conclusion from this review was that whilst studies have compared cortisol levels in melancholic and atypical depression, few if any studies have been carried out comparing cortisol secretion in healthy subjects and atypical depression.

It has to be recognized that one possible explanation as to why until now there has been no general consensus as to the status of cortisol levels in atypical and non-atypical MD is because there is wide variance in methods of assessment used. Methods to date have included blood sampling, urine collection or salivary analysis, with both basal and stimulated measures used. Furthermore, assessing the HPA axis can be problematic because cortisol is a pulsatile hormone and is released in stressful circumstances, such as blood sampling. Recent approaches have tried to use naturalistic and non-invasive measures, such as CAR in saliva (Bhagwagar et al., 2003; 2005). This method is able to yield information as to cortisol levels at the time of assessment, but it does not give an indication of levels of the cortisol in the past or over a long-term period. This problem could potentially be solved by using hair cortisol analysis (Russell et al., 2011). There is,

thus, accumulating evidence that hair cortisol could be a valid measure of long term HPA axis function, and an improvement upon current methods of assessment.

In summary, there is some supporting evidence that atypical and BD, along with CFS, show a different HPA axis signature to that of classical MDD. However, this needs further exploration with improved sampling methods such as hair analysis and careful assessment of confounding features such as early life stress

The novelty of this study also includes: 1) set up a new technique to assess HPA axis through hair sample specimens which reflects a better approach to assess chronic cortisol levels which is highly related to psychiatrics illnesses 2) obtain an improving correlation in chronic cortisol levels between saliva specimens (taking 6 samples per month/4 times= 24 specimens per subjects) and hair cortisol in four different psychiatrics illnesses and comparing with a control group. 3) Determine hypercortisolaemia and hypocortisolaemia levels in hair for these diseases. 4) solve several questions relate to clinic, neurobehavioural and neurobiology of these psychiatric illnesses that until now there are several controversies 5) assess the effect of different psychiatrics treatments over chronic cortisol levels and correlate them with the clinical outcome.

10.-Output and dissemination:

The results of this research will be disseminated through internal departmental scientific activities at the Institute of Psychiatry, King's College University, and conferences. Ultimately, the intention is to divulgate results by publishing data in research papers.

11.- Proposed Timetable:

Months 0-6: validate novel assays in laboratory. Write systematic review. Finalise protocols, and explore the possibility of recruitment from Brazil or Chile. Purchase and set

up of any relevant equipment for hair cortisol analysis, pilot or determine rough quantities of hair to be cut, pulverised and extracted.

Months 6-30. Patient recruitment.

Months 30-36: data analysis and write up.

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