ORIGINAL RESEARCH

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Human hair cortisol analysis: Comparison of the internationally- reported ELISA methods

Abstract

Purpose: Recently, hair cortisol has become a topic of global interest as a biomarker of chronic stress. Different research groups have been using different methods for extraction and analysis, making it difficult to compare results across studies. A critical examination of the reported analytical methods is important to facilitate standardization and allow for a uniform interpretation.

Methods: This study qualitatively compared four published procedures from laboratories in Germany, the Netherlands, USA and Canada. Multiple aspects of their procedures were compared.

Results: A major difference among the laboratories was the ELISA kit used: the Canadian laboratory used the kit from ALPCO Diagnostics (Salem, MA, USA), the American laboratory used the kit from DRG International (Springfield, NJ, USA), the German laboratory used the kit from DRG Instruments GmbH (Marburg, Germany), or IBL (Hamburg, Germany), and the Dutch used the kit from Salimetrics (Suffolk, UK). In addition, there are noted differences in hair mass used as well as washing and extraction procedures. The range of hair cortisol levels determined in healthy volunteers by the four groups was within 2.3-fold: Koren, 46.1 pg/mg; Van Rossum, 29.72 pg/mg; Kirschbaum, 20 pg/mg and Laudenslager ~ 27 pg/mg.

Conclusions: The relative similarities in hair cortisol values in volunteers among the four laboratories should facilitate a quality assurance exchange program, as a necessary step toward clinical use of this novel test.

Manuscript submitted 9th August, 2013

Manuscript accepted 23rd November, 2013

Clin Invest Med 2013; 36 (6): E312-E316.

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For decades now, hair analysis has gained an increasing role in detecting chronic exposure to drugs of abuse [1]. Because hair grows on average 1 cm per month, deposition of xenobiotics and endogenous compounds in hair, determined by sectioning a sample along the hair shaft, can serve as biomarkers of long term exposure to drugs [1]. Recently, hair cortisol has become a topic of global interest as a biomarker of chronic stress [2]. An increasing body of evidence has shown that hair cortisol levels reflect systemic plasma levels of the hormone over time, allowing estimation of chronic stress, both psychological and physiological, as well as disorders of the hypothalamic-pituitary-adrenal axis, including Cushing syndrome and Addison's disease [2-4].

Cortisol, determined through hair analysis, is the focus of increased scientific activity due to its contributions to stress-related psychobiological research, where there is a lack of other valid biomarkers for chronic stress. In general, different laboratories have been using a variety of techniques for measuring hair cortisol; however, in order to standardize these methods and facilitate their clinical use, existing differences in these procedures used require attention. While both GC/MS, and GC-MS-MS methods for assessing hair cortisol levels have been described [5,6], ELISA immunoassay methods are used worldwide to measure hair cortisol levels [2,7-14]. The objective of this review was to compare the methodologies used for hair cortisol analysis and identify areas that should be standardized as a first step toward introduction of hair cortisol as a biomarker in clinical settings.

Methods

Using Medline and Exerta Medica, international groups who have published consistently over the years on the measurements of hair cortisol were identified. Methodological aspects involved in ELISA for hair cortisol were compared among these groups, including the mass of hair used, washing methods, pulverization, extraction, temperature and centrifugation conditions, evaporation methods, the reconstituting solvent, duration of vortexing, the ELISA kits used for analysis and its reported cross reactivity, the wavelength used to quantify cortisol levels and, lastly, the mean cortisol levels in healthy volunteers reported by each research group.

Results

A total of four laboratories have been identified in Germany, the Netherlands, the USA and Canada. Although all four groups perform their analysis using ELISA methods, their methods differ in potentially important aspects.

Hair mass

The Van Rossum [11,12] and Koren [7] groups used 10-15 mg of hair. The Kirschbaum group used 25-50 mg in their first publications [8] and 10-15 mg in their recent studies [10] while the Laudenslager group [3] used 50 mg.

Washing method

The Kirschbaum and Laudenslager groups washed hair samples two or three times for 3 minutes each in 2.5 ml isopropanol. The Koren group washed hair samples using a similar method but in 3 ml isopropanol. The Van Rossum group did not utilize hair washing.

Pulverization

Koren and Van Rossum groups minced hair samples with surgical scissors. The Kirschbaum group pulverized samples for 5 minutes using a Retsch ball mill at 30 Hz and the Laudenslager group did the same but for 10 minutes at 25 Hz.

Extraction solvent

Three groups used 1 ml methanol for extraction while the Kirschbaum group used 1.5 ml methanol.

Duration of extraction and temperature

Koren and Van Rossum groups incubated the samples for 16 hours at 50°C and 52°C, respectively. Laudenslager and Kirschbaum groups incubated samples for 24 hours at room temperature.

Centrifugation during extraction

The Koren group centrifuged their samples at 100 rpm, The Van Rossum group shook their samples gently and the Kirschbaum group used slow rotation.

Solvent evaporation

All four laboratories use nitrogen to evaporate residual solvents, but they varied with respect to hot plate temperatures used; Koren and Van Rossum groups evaporated solvent from samples at 50°C under a steady stream of nitrogen for 12-15 min. The Kirschbaum group evaporated solvent from samples at 60°C for 20 min and the Laudenslager group at 38°C for 30 min.

Reconstituting solvent

Koren and Van Rossum groups reconstitute samples using 250 µl of phosphate buffered saline, while Laudenslager and Kirschbaum groups used 400 µl of the same buffer.

Duration of vortexing

Koren, Van Rossum and Laudenslager groups vortexed samples for 1 minute, initially within 30 seconds resuspension, while the Kirschbaum group vortexed the samples for 15 seconds.

ELISA used and cross reactivity

ELISA is a highly sensitive technique for detecting and measuring cortisol in a solution; the solution is run over a surface to which immobilized antibodies specific to cortisol have been attached, and if cortisol is present, it will bind to the antibody layer, and its presence is verified and visualized with an application of antibodies that have been tagged in some way. The steps of the ELISA result in a colored end product which correlates to the amount of cortisol present in the original sample [14]. Types of ELISA kits used by each group are as follows: The Koren group used a Salivary Cortisol Immunoassay Kit (ALPCO Diagnostics; Salem, MA, USA). The Van Rossum group used an ELISA Kit for Salivary Cortisol (DRG International, Springfield, NJ, USA) or ELISA Kit for Salivary Cortisol (DRG Instruments GmbH, Marburg, Germany). Kirschbaum used a Cortisol Saliva Luminescence Immunoassay (IBL-Hamburg-Germany). Lastly, the Laudenslager group used a Salivary Cortisol Enzyme Immunoassay (Salimetrics Europe, Suffolk, UK).

Cross reactivities for cortisol by the different ELISA kits used are presented in Table 1. In general, while cross reactivities are different among the kits, their potential effects on measured cortisol levels are minimal, as the cross reacting molecules are rarely on board and are not used concomitantly

Centrifugation during ELISA

The Koren group used 100 rpm while the Laudenslager group used 500 rpm. No information was reported by the other two groups.

Wavelength while measuring cortisol

All groups quantified their cortisol samples at 450 nm. The quantification is from a comparison to a standard curve.

Cortisol range determined

The healthy control group cortisol levels range obtained from multiple studies by the four groups fall in the same range: The mean reported hair cortisol levels are 46.1 pg/mg for the Koren group; 29.72 pg/mg for the Van Rossum group, 27 pg/mg for the Laudenslager group and 20 pg/mg for the Kirschbaum group.

Discussion

The present study compared, for the first time, the analytical techniques used for determination of human hair cortisol. Investigating all aspects of this methodology is a necessary first step toward creating international clinical standards for this new and exciting test, to ensure precision and reproducibility.

Despite variability in the different steps used by the four groups, it appears that the resultant measures of hair cortisol are similar (less than or equal to a 2.3-fold difference) in healthy volunteers. Each research group used different control subjects, and hence, it is possible that the differences among laboratories due to methodological differences alone are smaller. To use this powerful biomarker in clinical settings, to evaluate both stress and disorders of the hypothalamus-hypophyseal-adrenal axis, a methodological comparison would have to be performed, with the different laboratories receiving similar samples and analyzing them anonymously. In parallel, the same sample of hair should be assessed by the different ELISA methods in a single laboratory. Such a program was initiated during the Toronto meeting of the Society for Hair Testing on June 27, 2012 and the process is ongoing.

In summary, the ability of hair cortisol measurements to detect stress associated with a wide range of conditions such as pain, psychiatric morbidity and myocardial infarction, as well as Cushing syndrome and Addison's disease, holds significant clinical promise for new diagnostic tests. For this to happen, the existing tests will have to be standardized through an international quality assurance program, which is now in progress.

Acknowledgments

Wed Albar is sponsored by the Saudi Cultural Bureau in Canada. No additional external funding was obtained for the conduct of this work.

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TABLE 1. Comparison of cross reactivities among the four ELISA kits.

Kirschbaum ^a	
Compound	Cross-reactivity [%]
Cortisol, hydrocortisone	100
Prednisolone	57
11-Deoxycortisol	12
Corticosterone	2.5
Cortisone	2
Prednisone	1
17 α -Hydroxyprogesterone	0.5
Deoxycorticosterone	0.3
6 α -Methyl-17 α -Hydroxyprogesterone	0.1
Progesterone	<0.05
Dexamethasone	<0.05
17 α -Hydroxypregnenolone	<0.01
Dehydroisoandrosterone	<0.01
Androstenedione	<0.01
Oestriol	<0.01
6 α -Methyl-17 α -Hydroxyprogesteroneacetate	<0.01
Pregnenolone	<0.01
Oestrone	<0.01
Testosterone	<0.01
17 α -Hydroxyprogesterone-17 sulphate	<0.01
Androsterone sulphate	<0.01
Testosterone sulphate	<0.01
Cholesteryl sulphate	<0.01
17 β -Oestradiol-17 sulphate	<0.01
DHEA-S	<0.01
Koren ^c	
Steroid	%Cross Reactivity
Cortisol	100
Prednisolone	13.6
Corticosterone	7.6
Deoxycorticosterone	7.2
Progesterone	7.2
Cortisone	6.2
Deoxycortisol	5.6
Prednisone	5.6
Dexamethasone	1.6

Laudenslager ^b		
Compound	Spiked Concentration (ng/mL)	% Cross-reactivity in HS Salivary Cortisol EIA
Prednisolone	100	0.568
Prednisone	1000	ND
Cortisone	1000	0.130
11-Deoxycortisol	500	0.156
21-Deoxycortisol	1000	0.041
17 α -Hydroxyprogesterone	1000	ND
Dexamethasone	1000	19.2
Triamcinolone	1000	0.086
Corticosterone	10,000	0.214
Progesterone	1000	0.015
17-Estradiol	10	ND
DHEA	10,000	ND
Testosterone	10,000	0.006
Transferrin	66,000	ND
Aldosterone	10,000	ND

ND = None detected (<0.004)

Van Rossum ^d	
Steroid	% Cross reaction
Cortisol	100
Corticosterone	29.00
Cortisone	3.00
11-Deoxycortisol	< 1.00
17-OH Progesterone	<0.50
Prednisone	< 0.10
Progesterone	< 0.10
Dexamethazone	< 0.10
Desoxycorticosterone	< 0.10
Dehydroepiandrosterone sulfate	< 0.10
Estradiol	< 0.10
Estriol	< 0.10
Estrone	< 0.10
Testosterone	< 0.10

^a. Immuno-Biological Laboratories, Hamburg, Germany^b. Salivary Cortisol Enzyme Immunoassay, Salimetrics, Suffolk, UK^c. ALPCO Diagnostics; Salem, USA^d. DRG® Salivary Cortisol ELISA DRG International, city, USA

* Letters correspond with kind of kit used by all groups.

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