Lessons from rare diseases: pathophysiology of stress-related diseases and organization and evaluation of care for patients with Cushing's syndrome

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CHAPTER 4

HAIR CORTISOL CONCENTRATIONS IN CHRONIC CENTRAL SEROUS CHORIORETINOPATHY

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ABSTRACT

Purpose

Central serous chorioretinopathy (CSC), a distinct form of macular degeneration, has been associated with glucocorticoid use, and possibly also with an increased endogenous activity of the hypothalamic-pituitary-adrenal (HPA) axis. To estimate long-term glucocorticoid exposure, measurement of hair cortisol concentrations (HCC) have emerged. This cross-sectional study aimed to investigate HCC, as a reflection of chronic endogenous steroid exposure, in a cohort of chronic CSC patients (cCSC).

Methods

HCC were determined in 48 cCSC patients and 230 population-based controls (Lifelines cohort study), not using exogenous corticosteroids.

Results

Increased HCC (defined as > 10.49 pg/mg) were present in 2 (4%) cCSC patients and 13 (6%) controls. Mean HCC values were not different between patients and controls, and no difference in HCC were found between patients with active cCSC disease and patients with inactive disease. No correlation between HCC and urinary free cortisol (UFC) levels in cCSC patients was found.

Conclusions

This study shows that HCC in cCSC patients are not elevated compared to population-based controls, and no association between HCC and cCSC severity was found. This finding questions the previous suggestion that cCSC is associated with increased HPA axis activity. In line, HCC do not seem useful in monitoring cCSC disease activity.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a specific chorioretinal disease, in which choroidal hyperpermeability and retinal pigment epithelium damage occurs, leading to serous subretinal fluid accumulation.[1-3] When persistent and left untreated, irreversible loss of vision occurs, resulting in a decreased quality of life.[4, 5] Although the pathogenesis of CSC is currently unclear, biochemical stress in the form of both exogenous steroids as well as endogenous hypercortisolism have been reported in association with CSC.[1, 6, 7] Recently, we have reported an increased activity of the hypothalamic-pituitary-adrenal (HPA) axis based on increased 24 hour urinary free cortisol (UFC) excretion, albeit still within the normal cortisol range, and without disruption of circadian rhythm.[8] Although some of our cases of Cushing's syndrome have presented with CSC,[6] in this consecutive series we did not diagnose a single new case of Cushing's syndrome during screening of a large cohort of chronic CSC (cCSC) patients.[8]

The activity of the HPA axis as a proxy of endogenous exposure to stress can be evaluated with a number of tests, all reflecting different aspects and periods of endogenous cortisol exposure: 24 hour UFC levels reflect cortisol exposure during one day, whereas plasma and salivary cortisol levels provide information on the extent of cortisol present at a certain moment in time and its diurnal variation. To estimate long-term glucocorticoid exposure, measuring cortisol concentrations in scalp hair (hair cortisol concentrations (HCC)) has emerged over the past years. Scalp hair grows approximately 1 cm a month at a relatively stable rate, and steroid hormones are shown to retain in hair,[9, 10] making hair useful for the estimation of glucocorticoid exposure over a period of months.[11] Cushing's syndrome, obesity, cardiovascular disease, metabolic syndrome, and psychopathology have previously been associated with increased HCC,[11-13] and in patients with other ophthalmological diseases such as progressive keratoconus, elevated hair cortisol levels have been reported.[14] Recently, a small pilot study including 11 patients showed increased HCC in patients with active CSC.[15]

In the present study, we evaluated HCC in a large cohort of cCSC patients. In order to further investigate the suspected relationship between cCSC and cortisol as a measure for HPA axis activity, patients data were compared to the HCC of adult controls from the general population.

MATERIALS AND METHODS

Study design

Cross-sectional study in cCSC patients. The key objective was to assess HCC as a measure for the long-term endogenous cortisol exposure in these patients. For this purpose, HCC of patients with cCSC were compared to HCC of a population-based control group. In addition, a clinical evaluation of the patients took place on the outpatient clinic of the Division of Endocrinology of the Leiden University Medical Center. The relation between HCC and UFC was evaluated in cCSC patients. Written informed consent was obtained from all participants, and approval of the institutional review board and the ethics committee was obtained (NL50816.058.14). Research was conducted following the tenets of the Declaration of Helsinki.

Study population

Patients

Of the adult patients with cCSC who were followed at our tertiary referral center, 86 consecutive patients were invited to participate. The diagnosis of cCSC had been confirmed according to current standards (i.e. fundoscopy, digital colour fundus photography (Topcon Corp., Tokyo, Japan), fundus autofluorescence (Spectralis Heidelberg retinal angiography (HRA) + optical coherence tomography (OCT); Heidelberg Engineering, Heidelberg, Germany), spectral-domain OCT (Spectralis HRA + OCT), fluorescein angiography (Spectralis HRA + OCT) and indocyanine green angiography (Spectralis HRA + OCT)).[1, 2, 16-19] For inclusion, the following characteristics had to be present on multimodal imaging within the past two years: serous subretinal fluid on OCT, and either ≥ 1 area of irregular retinal pigment epithelium window defects or multifocal diffuse leakage on fluorescein angiography. Patients were divided in subgroups of either active or nonactive cCSC at the moment of HCC evaluation, in which active disease was defined by subretinal fluid presence. Patients diagnosed with acute CSC were excluded, defined by a smoke stack pattern of a focal leakage spot on fluorescein angiography,[1, 2, 16-19] as well as patients with evidence for another retinal diagnosis.

All cCSC patients participated in the study on endocrine phenotyping of the HPA axis as mentioned above (n=86),[8] as well as in a psychological questionnaire survey (n=86, data presented elsewhere).[20] For the present study, exclusion criteria were excessive alcohol intake (>21 units/week), the use of corticosteroids (both systemic as well as local) or sleep medication prior to the development or during the time-course of cCSC, and either night shift work or travelling from another time zone in the six weeks prior to evaluation. Endocrine evaluation of the patients consisted of a detailed medical history, a complete physical examination, specifically aimed to detect subtle signs of Cushing's disease, and blood, urine, and saliva analysis (data presented elsewhere)[8]. The collection of scalp hair succeeded in 48 patients. In the other 38 patients, hair collection failed due to the absence of at least 1 cm of hair (n=33), or due to the absence of patients' permission to cut hair (n=5). After reassessment of the retinal imaging by two independent ophthalmologists, two of the cCSC patients were considered to have less typical findings on imaging, and were excluded in a sensitivity analysis.

Population-based controls

Control data were derived from Lifelines, a multi-disciplinary prospective population-based cohort study examining in a three-generation design the health and health-related behaviours of 167,729 persons living in the North of The Netherlands (www.lifelines.nl). It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics.[21]

For the present study, data on HCC collected for a previously described study were used.[22] For this preceding research, approved by the Medical Ethics Review Committee of the University Medical Center Groningen, 295 adult participants of Lifelines were included in November and

December of 2013. Written informed consent was provided by all participants. The participants came for a study site visit including measurements of vital parameters and anthropometry, a fasting venepuncture, and scalp hair collection, of which the results were presented by Wester et al.[22] HCC were successfully determined in 266 participant samples. From this cohort, participants using systemic (n=3) or local glucocorticoids (n=33) were excluded for the present analysis.

Hair processing and analysis

During the study visits, a sample of scalp hair of approximately 100-150 hairs from the posterior vertex was cut, as close to the scalp as possible. The hairs were taped to a paper, and stored at room temperature in the dark in envelopes until further processing. Hair samples, both from the cCSC patients as well as from the controls, were processed and analysed as was described previously.[10] In controls, approximately 20 mg of the proximal 3 cm (if present) of each hair sample was weighed, and cut into 1 cm segments; an average of the 3 HCC was used for analysis. In patients, only the most proximal cm of hair was used for the measurement of HCC. The samples were washed for 2 minutes in 2 mL of liquid chromatography – mass spectrometry (LC-MS) grade isopropanolol, and left to dry. The hairs were extracted for 18 hours at 25° centigrade in 1.4 mL LC-MS grade methanol and 100 µL of internal standard. Solid phase extraction was used to purify the extracted samples, and quantification of cortisol was performed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) using a Xevo TQ-S system (Waters, Milford, MA, USA). Increased HCC were defined as > 10.49 pg/mg, as described by Wester et al. [22]

Statistical analysis

SPSS Statistics version 23 was used for statistical analysis (IBM Corp., Armonk, NY, USA). Data were presented as mean and standard deviation (SD), unless mentioned otherwise. Hair cortisol concentrations were logarithmically transformed to achieve a normal distribution. Data were analysed using independent sample *t*-tests. Analyses were stratified according to gender. The groups were compared using a linear regression model, correcting for potential confounders such as duration of cCSC disease and age, since hair cortisol levels were shown to increase with age.[22] A *P* value below 0.05 was considered statistically significant. A post hoc sensitivity analysis excluding the two less typical cCSC patients was performed. Moreover, a sensitivity analysis excluding outliers (n = 2 control, no patients) was completed, using an outlier test (Rosner's Extreme Studentized Deviate test) to determine significant outliers. The correlation between HCC and UFC levels was assessed using Pearson's correlation.

RESULTS

Baseline characteristics

Forty-eight cCSC patients (41 males [85%]) and 230 population-based controls (63 males [27%]) were included (Table 1). The gender distribution in cCSC patients was in line with the currently available literature.[1, 19] At the time of evaluation, the mean duration of cCSC disease since diagnosis had been established by an ophthalmologist was 3.9 years (range 0.2-33.0). Active cCSC (i.e. presence

of subretinal fluid) was present in 31 patients (65% of the patients). With a mean age of 49.2 years (range 33-72), cCSC patients were 7 years older than controls (mean age 42.2 years, range 18-85, *P*<0.01). No cases of Cushing's syndrome according to conventional tests were present.[8]

Hair cortisol concentrations (HCC)

Hair cortisol concentrations ranged from 0.6 to 20.8 pg/mg in cCSC patients and from 0.7 to 79.8 pg/mg in controls. Increased HCC, i.e. > 10.49 pg/mg, was present in 2 cCSC patients (4%) and 13 controls (6%).

Mean HCC in male cCSC patients were 3.9 (SD 3.7) compared to 4.6 (4.7) in male controls, *P*=0.32. In females, mean HCC were 4.3 (4.3) in cCSC patients and 3.5 (7.1) in controls, *P*=0.60 (Figure 1). Also, after correction for age, no significant differences in HCC between the cCSC patients and controls were found (*P*=0.14 males, *P*=0.08 females). Likewise, correction for duration of cCSC disease did not change the results (males *P*=0.72, females *P*=0.89).

Patients with active cCSC had mean HCC of 3.8 (2.9), and in patients with inactive disease mean HCC of 4.2 (5.0) were found, *P*=0.86 (Figure 2).

In 47 of the 48 cCSC patients (98%) UFC levels were measured. UFC ranged from 19 to 274 nmol/24 hour (mean 84.0 (44.2)). Figure 3 shows the absence of a correlation between HCC and UFC levels in cCSC patients (R^2=0.07, *P*=0.63).

The exclusion of two atypical cCSC patients did not affect any of the described results. Also the exclusion of the significant outliers in HCC did not change the aforementioned results (data not shown).

DISCUSSION

This study revealed that HCC in patients with cCSC were not different when compared to population-based controls. In addition, no differences in HCC were found between patients with active cCSC disease and patients with inactive disease. Hence, our study demonstrates that HCC are not useful in monitoring cCSC disease activity. No correlation between HCC and UFC was found in cCSC patients either.

To our knowledge, this is the first study evaluating HCC in a relatively large cohort of cCSC patients. The only study published to date involved a pilot study investigating HCC in a very small group of 11 patients with either active acute or chronic CSC, which showed, in contrast to

Table 1. Clinical characteristics of participants.

	cCSC patients *n=48*	**Controls** *n=230*	**p value**
Age, yrs (mean, SD)	49.2 (9.5)	42.2 (11.6)	<0.01
Sex, male/female	41/7	63/167	<0.01
Duration of cCSC disease, yrs (median, range)	0.9 (0.2–33.0)	-	-

Data are presented as mean (SD), median (range) or as numbers. cCSC = chronic central serous chorioretinopathy, yrs = years.

our study, increased HCC in these patients.[15] The pilot study showed mean age-adjusted HCC of 20.1 pg/mg in CSC patients and 11.1 pg/mg in healthy controls, compared to our 3.9 pg/mg in male cCSC patients and 4.6 pg/mg in controls. However, the size of the pilot study makes the results susceptible to sampling and selection bias, and the heterogeneity of the patients stands in the way of generalizability. Our study included 48 consecutive patients with only chronic CSC, making the results valid and generalizable to this patients category. Moreover, the pilot study used an immunoassay for the determination of HCC, whereas LC-MS/MS measurements were used in the present study. Immunoassays are shown to differ in steroid crossreactivity depending on the assay used, and are described to measure substantially higher HCC with a greater variation than the more accurate LC-MS/MS based methods.[23] The absence of a correlation between HCC and UFC in our cCSC patient population is in line with a previously published evaluation of HCC in combination with UFC in healthy controls.[24] However, in patients with Cushing's syndrome and corresponding pathological cortisol excess, strong correlations between HCC and UFC have been reported.[25, 26] The proposed relationship between cortisol, both endogenous as well as exogenous, and CSC has been widely described.[1, 6, 7] The pathophysiology, however, remains to be elucidated, although several underlying mechamisms have been hypothesized. Platelet aggregation is increased by endogenous hypercortisolism, leading to increased blood viscosity and microthrombi.[27] Hyperpermeability and choroidal fragility have also been associated with hypercortisolism,[28] and an increased expression of adrenergic receptors has been correlated with corticosteroids.[29] Previous animal studies have suggested that mineralocorticoids play a pathophysiological role,[19, 30] by activating the mineralocorticoid receptor in choroidal endothelial cells, leading to choroidal vasodilation.[19] Moreover, the possible pathogenetic effect of activating

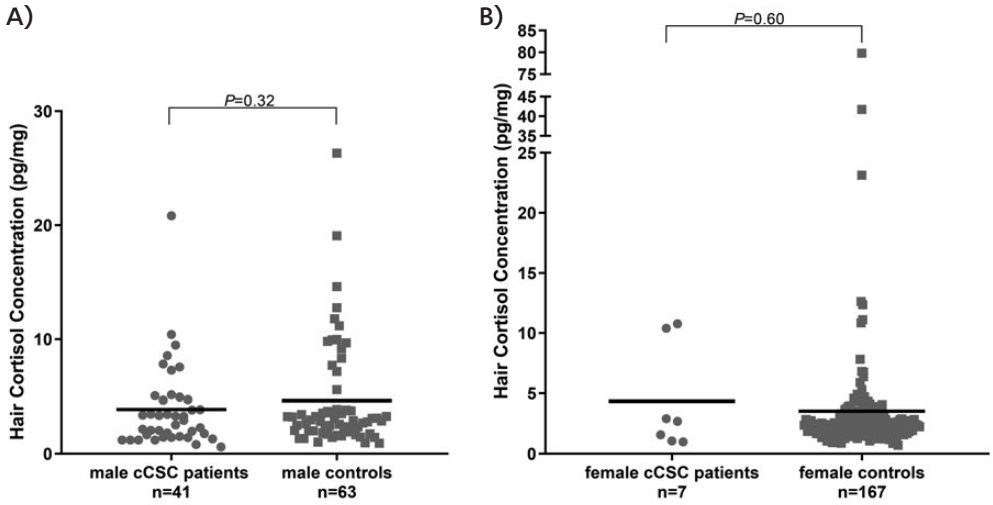

Figure 1. Hair cortisol concentrations (HCC) in cCSC patients and population-based controls stratified by sex. **A**, Males. **B**, Females. Data presented as individual values and mean. cCSC = chronic central serous chorioretinopathy, HCC = hair cortisol concentrations.

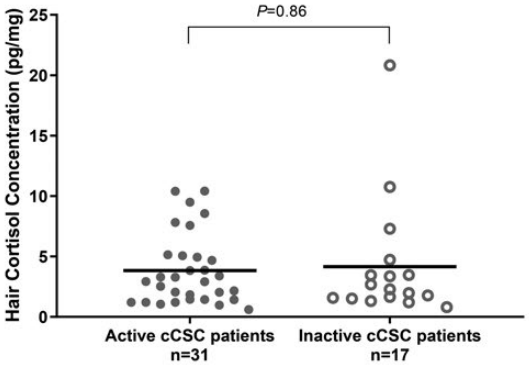

Figure 2. Hair cortisol concentrations (HCC) in cCSC patients with active disease and cCSC patients with inactive disease. Data presented as individual values and mean. cCSC = chronic central serous chorioretinopathy, HCC = hair cortisol concentrations.

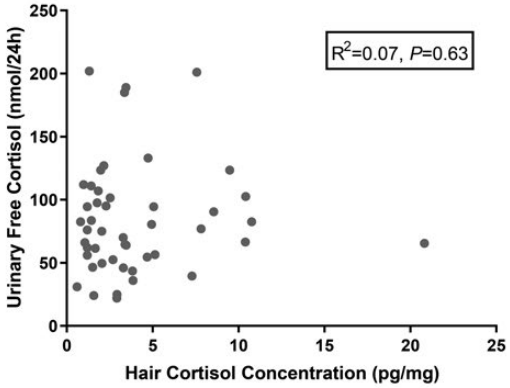

Figure 3. Correlation between HCC and UFC in patients with cCSC. Data presented as individual values. N = 47 cCSC patients. cCSC = chronic central serous chorioretinopathy, HCC = hair cortisol concentrations, UFC = urinary free cortisol.

the mineralocorticoid receptor may be modulated by several genetic receptor variants.[31] With regard to the biological evaluation of patients using the clinically available screening tests for cortisol, we recently reported significantly higher 24 hour UFC levels in cCSC patients, albeit within the normal reference range, with preservation of normal diurnal rhythmicity.[8] In the light of clinical cortisol testing with HCC as a measure for long-term cortisol exposure, the current analysis does not show increased HCC in cCSC patients. We propose that either the HCC technique is not sensitive enough to detect minor and perhaps short-term elevations in cortisol concentrations within a normal range, keeping in mind that a wide individual variation in normal cortisol levels and glucocorticoid sensitivity. Altered glucocorticoid sensitivity due to glucocorticoid receptor gene polymorphisms has been shown to modify manifestations of several diseases.[32-35] Or perhaps these minor increases in cortisol concentrations on a tissue level leading to cCSC specific alterations in choroid and retina

are not reflected by increased cortisol concentrations in hair. On the other hand, based on our findings, one could also postulate that the long-term exposure to cortisol is not increased in cCSC. Perhaps a short peak or a prolonged temporary elevation in cortisol levels is sufficient to induce pathological alterations in the choroid and/or retina, and may have anticipated the current chronic status. We cannot rule out that accidentally non-reported exogenous corticosteroid use may have lowered the HCC in the control group. However, since this was extensively interrogated, we believe the potential effect of non-reported corticosteroids to be limited. An alternative explanation for the absence of increased HCC in cCSC patients, is that the relationship between cortisol and cCSC is not as straightforward as suggested so far.

Our study also has limitations. The cross-sectional character does not allow drawing conclusions on any (absence of a) causal relationship. In controls, the proximal 3 cm of each hair sample was cut into 1 cm segments, and an average of the 3 HCC values was used for the current analysis. Since our patient population consisted mainly of men with most of them having short hair, HCC were only measured in the proximal 1 cm of hair. However, since Noppe et al described that the HCC decline gradually from proximal to more distal hair segments,[10] this would imply higher HCC in controls when only the most proximal cm of hair had been used, resulting in an even smaller difference between female cCSC patients and controls. Last, with our choice to stratify the study groups for analysis, potential residual confounding was introduced. Yet, because stratification on only gender (i.e. two strata) was applied, we consider the effect of this stratification negligible.

In conclusion, HCC as a clinical measure for long-term cortisol exposure in cCSC patients are not elevated when compared to population-based controls. In addition, no difference in HCC were found between different cCSC disease stages. Therefore, the results of this study argue against the use of HCC in monitoring cCSC disease activity. Further research unravelling the role of cortisol and the stress axis in the pathophysiology of cCSC is required.

CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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AUTHORS CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work, and approved it for publication. FH, ED, and MS collected the data. FH wrote the paper and designed the figures. AP, CB, NB, OD, ER, MS, JB, and GD have made substantial contributions to the concept and design of the work and interpretation of data. All evaluated the paper.

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