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Hair cortisol concentrations in decedents with severe mental illness – An autopsy-based cohort study

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ABSTRACT

Severe mental illness (SMI) is associated with a marked increase in mortality, both from natural and unnatural causes. Patients may be subject to accelerated aging and an increased disposition for developing chronic somatic disease. One possible contributory cause to this may be chronic hyperactivity of the hypothalamic-pituitary-adrenal (HPA)-axis and subsequent increased levels of cortisol. This study analyzed hair cortisol concentrations (HCC) using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) in hair samples from forensic autopsies of patients with SMI, validated via health registry data. Results: HCC was not associated with a particular diagnosis or increased in patients compared with decedents without SMI. HCC was higher in decedents who had been prescribed psychotropic medication, especially SSRIs and benzodiazepines. HCC was not associated with a history of coerced admissions or high number of days of admission. Future studies evaluating HCC as a proxy marker for stress should consider including medication history as a covariate. Reference ranges for healthy adults using standardized methods are needed in order to provide better interpretation of patient HCC.

1. Introduction

Severe mental illness (SMI) constitutes a major burden of health [1], it is associated with an all-cause mortality twice as large as in the background population, and a median 10 year decrease in life expectancy [2]. The increased mortality is seen for death from both natural and unnatural causes (i.e. accidents, suicide and homicide) [3,4]. There are several proposed reasons for this excess morbidity and mortality [4], including side effects to pharmacological treatment, e.g. weight gain associated with antipsychotics [5], a more unhealthy lifestyle [6], reduced effect of prophylaxis and treatment of cardiovascular disease [7] and cancer [8], and up to three times increased rates of addiction to alcohol and other harmful substances [9]. There are also indications that patients with SMI may be subject to accelerated aging, giving them a disposition for earlier development of chronic somatic disease compared with the general population [4,10,11].

Part of the morbidity gap may be a result of the stressful nature of a SMI, leading to chronic hyperactivity of the hypothalamic-pituitary-adrenal-axis (HPA-axis) and neuroimmune pathways [12]. Therefore, cortisol has been studied extensively as a possible biomarker for stress levels due to SMI. However, previous studies have reported conflicting results; some finding increased levels of cortisol in depression and schizophrenia [13–17], some reporting lower hair cortisol concentration (HCC) in patients [18,19], and others reporting no difference between patients and controls [20–22]. Comparison of these studies is complicated by the fact that illness severity and specific symptomatology (as opposed to presence of a diagnosis alone) [19,23], timing of sampling (i.e. at illness debut vs. chronic stable illness vs. remission) [23], neuropharmacological treatment [23,24] as well as subject age influence cortisol levels [22,25].

The possibility of performing cortisol measurements in hair samples represent significant advantages compared to sampling in urine, blood or saliva, including being less invasive, pain-free and representative of the average plasma levels of corticosteroids for time periods of weeks to months, and thus not liable to be influenced by diurnal variation in endogenous cortisol release [26–29].

In this study, we investigated whether HCC in a population of 107 decedents, who were referred for a forensic autopsy and who had a known or suspected history of mental illness, was associated with diagnosis, history of long or coerced admissions to psychiatric wards, as a proxy measure for illness severity, or treatment with psychotropic medication.

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2. Materials

The sample population consisted of 107 individuals who underwent autopsy at the Department of Forensic Medicine, University of Copenhagen, between May 2015 and October 2017. The population is a subgroup from SURVIVE, a national, prospective, autopsy-based cohort study focusing on excess morbidity and mortality in patients with severe mental illness [30]. Criteria for inclusion were either known or suspected history of mental illness of any type, or evidence of treatment with any kind of neuropsychiatric medication. Individuals were excluded if there was pronounced decomposition, defined as greenish discoloration anywhere other than the abdominal skin, if their hair length was under 1 cm, or if the suspected manner of death was homicide.

At inclusion, all cases were grouped tentatively as having a history of either schizophrenia, bipolar disease, depressive disorder or another psychiatric illness than these. Diagnoses were subsequently corroborated using registry data from the Danish National Patient Registry. We also retrieved data concerning prescriptions for any neuropsychiatric medication and medication containing corticosteroids, data concerning type and duration of all psychiatric hospitalizations and data about whether hospitalizations were coerced. Prescription data only covers out-of-hospital treatment, not during hospitalization. All registry data covered the period from January 1st 1995 until death.

In order to define mutually exclusive patient groups, we grouped all individuals who had multiple types of psychiatric diagnoses hierarchically in accordance with the International Classification of Diseases, 10th edition (ICD-10), chapter 5. Thus, schizophrenia diagnoses (F20.0-F20.9) were prioritized over bipolar disease (F30.0-F31.9), which was prioritized over depressive disorder (F32.0-F33.9), which was prioritized over any other psychiatric diagnosis, excluding dementia diagnoses (F00-F09). A single occurrence of a psychiatric diagnosis in relation to admission at a psychiatric ward was considered sufficient to include a case in one of the diagnostic groups. Decedents who did not have any registered psychiatric diagnosis served as controls. We identified 29 cases of schizophrenia (SCZ), 5 cases of bipolar disease (BIP), 12 cases of depressive disorder (DEP), 30 cases with another psychiatric diagnosis apart from these (OTH) and 31 cases with no lifetime history of admission to a psychiatric ward (CON).

Hair samples were collected as part of the standard autopsy procedure, with approximately 100 hairs cut from the posterior vertex, as close to the skin as possible. Hair samples were carefully placed in specialized containers, consisting of an inner wrapping of aluminum foil around the hair, to prevent individual hairs from displacing in the longitudinal direction, stored in cardboard envelopes at room temperature until analysis.

The study protocol was approved by the Danish National Committee on Health Research Ethics (ref. no. 1703611) and the Danish Data Protection Agency (ref. no. SUND-2016-06) and was conducted in accordance with Declaration of Helsinki. Consent for use of hair samples was obtained from the nearest next-of-kin in a standardized fashion, designed to be minimally obtrusive, as described previously [30].

3. Methods

Hair sample preparation and cortisol measurements were performed using a method developed and validated at our laboratory [31]. In brief, the hair samples were cut in 2 cm long segments from the proximal end, representing approximately two months of hair growth each. The first and second hair segments (S1 and S2, respectively) were analyzed for cortisol concentration. Ten mg of hair was washed with a sequence of isopropanol, water, water, and finally isopropanol. The second aqueous wash fraction was analyzed for possible external contamination, defined as the wash fraction containing 20 % or more of the content of the hair segment. The hair sample was then dried at room temperature overnight. The following day, the sample was suspended in methanol and pulverized using a bead homogenizer, followed by incubation at 37 °C for four hours. The methanol was evaporated, and then the remaining residue was reconstituted in mobile phase. The hair extract was filtered before being analyzed. A Waters ACQUITY UPLC system was used for chromatographic analysis and a Waters Xevo TQ-S tandem quadrupole mass spectrometer was used for detection. The method was continuously evaluated using control hair samples. The limit of quantification of cortisol in hair was 0.5 pg/mg. Intra- and interday precision were acceptable with a maximum relative standard deviation of 13.5 %. Interlaboratory comparison showed high consistency of our analysis results with those of other laboratories. Determinations of HCC were performed in duplicates and reported as pg/mg.

Reference ranges for HCC vary greatly between published studies. Upper limits of concentrations considered in the normal range have been reported as being up to 9.54 pg/mg [32] and 69.5 pg/mg [33] in children and adolescents, and 72.2 pg/mg [34], 91 pg/mg [27], 204 pg/mg [35], 500 pg/mg [25] and even 1500 pg/mg [21] in adults. These upper ranges also depend on the applied analysis method, with immuno-assay-based studies tending towards higher reference values than mass-spectrometric methods [21,25,33,35]. We chose to run analyses using two scenarios, the first including all measured concentrations, the other including only values equal to or below 91 pg/mg, the upper range of values detected in a published sample of 44 healthy volunteers using UPLC-MS/MS [27]. We found no difference in results and thus we report the results for the full dataset.

We analyzed prescription registry data for both the whole dataset (data available since January 1st 1995) and in the six months preceding death, with the reasoning that HCC would mainly be influenced by recent life events. The measured HCC and the total duration of admission to psychiatric wards did not assume normal distributions. Therefore we have log-transformed these values before statistical analysis. Reported values are anti-logged.

4. Results

Characteristics of the study population are listed in Table 1. There were no statistically significant differences of mean age, body height or in the distribution of gender. One-way analysis of variance showed statistically significant differences in body weight and body mass index across groups, but post-hoc Tukey test for standardized difference of means showed no significant difference of means between any pair of groups. The proportion of cases having a prescription for antipsychotics in the 180 days preceding death was highest in the schizophrenia group (65.5 %), the proportion of prescriptions for antidepressants and benzodiazepines were highest in the depression group (66.7 %). 58.1 % of the control group had a prescription for at least one psychotropic medication at one point in their life, despite having never been admitted to a psychiatric ward.

There were significantly more decedents who had experienced at least one episode of coerced admission to a psychiatric ward in their lifetime in the groups with bipolar disease and schizophrenia (40.0 % and 41.1 %, respectively), compared with other groups (0–13.3 %). The median total number of days admitted to a psychiatric ward was significantly longer in two groups with diagnoses of bipolar disease and schizophrenia compared to the group with depressive disorder and other psychiatric illnesses. By definition, the control group had no registered admissions to a psychiatric ward.

Age showed highly significant positive correlation with HCC, female gender was associated with higher HCC, though not statistically significant (p = 0.053). We provide p-values for both an unadjusted model and a model adjusted for gender and age.

Hair segment 1 (S1) was analyzed for all 107 cases, segment 2 (S2) was obtained and analyzed in 92 cases, with missing cases being due to hair length shorter than 2 cm. The highest measured HCC was 1100 pg/mg. 15.9 % of all measured values were above 91 pg/mg. Cortisol levels in
Table 1
Demographic characteristics of diagnosis groups and treatment history (prescriptions and admissions). Difference of means tested with one-way analysis of variance. Difference of proportions tested with Fisher’s exact test. BIP: bipolar disorder; DEP: depressive disorder; SCZ: schizophrenia; OTH: other psychiatric diagnosis; CON: control group (no psychiatric diagnosis according to registry data). Drug classes followed in parentheses by anatomical therapeutical chemical (ATC) classification codes used for registry lookup.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>BIP (5)</th>
<th>DEP (12)</th>
<th>SCZ (29)</th>
<th>OTH (30)</th>
<th>CON (31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD)</td>
<td>65.4 (11.1)</td>
<td>46.4 (16.5)</td>
<td>51.1 (11.5)</td>
<td>48.3 (16.3)</td>
<td>56.7 (20.1)</td>
</tr>
<tr>
<td>Male/Female (n)</td>
<td>2:3</td>
<td>6:6</td>
<td>12:17</td>
<td>14:16</td>
<td>18:13</td>
</tr>
<tr>
<td>Mean body height (SD)</td>
<td>168 (11)</td>
<td>169 (7)</td>
<td>171 (9)</td>
<td>168 (9)</td>
<td>172 (11)</td>
</tr>
<tr>
<td>Mean body weight (SD)*</td>
<td>59.2 (14.6)</td>
<td>81.3 (16.4)</td>
<td>80.8 (27.9)</td>
<td>66.3 (12.6)</td>
<td>72.8 (15.1)</td>
</tr>
<tr>
<td>Mean BMI (SD)**</td>
<td>20.6 (2.9)</td>
<td>28.6 (5.7)</td>
<td>27.4 (8.5)</td>
<td>23.6 (3.9)</td>
<td>25.0 (3.9)</td>
</tr>
</tbody>
</table>

History of prescription with any type of (proportion of group):

| Antipsychotic (N05A-*) | 100 % | 91.7 % | 100 % | 63.3 % | 19.4 % |
| Antidepressant (N06A-*) | 80.0 % | 100 % | 65.5 % | 70.0 % | 45.2 % |
| Benzodiazepine (N05BA, N05CD and N05CF) | 100 % | 83.3 % | 86.2 % | 73.3 % | 61.3 % |
| None of the above | 80.0 % | 83.3 % | 55.2 % | 60.0 % | 41.9 % |

History of prescription during the 180 days before death with any type of (proportion of group):

| Antipsychotic | 60.0 % | 50.0 % | 65.5 % | 30.0 % | 9.7 % |
| Antidepressant | 80.0 % | 66.7 % | 31.0 % | 46.7 % | 12.9 % |
| Benzodiazepine | 40.0 % | 66.7 % | 38.0 % | 40.0 % | 35.5 % |
| None of the above | 20.0 % | 25.0 % | 13.8 % | 26.7 % | 9.7 % |

Psychiatric admissions:

| Proportion with lifetime history of at least 1 coerced admission to a psychiatric ward*** | 40 % | 0 % | 41.4 % | 13.3 % | N/A |
| Median total days of lifetime psychiatric admissions (range)*** | 468 (22–1490) | 50.5 (7–212) | 666 (50–4102) | 20 (1–215) | N/A |

segments 1 and 2 showed high correlation (Pearson’s correlation coefficient of 95.6).

Contamination of the second aqueous wash fraction was present in 11 S1-segments and 5 S2-segments, for a total of 16 out of 199 samples (8 %).

Table 2 shows median HCC and mean log(HCC) of cases with wash contamination compared to cases without. We found no significant difference in mean log(HCC) between individuals with a prescription for a corticosteroid-containing drug or ointment and those without a prescription (data not shown). As there was no significant association between HCC and wash contamination, we chose to include the full data set in further analyses.

Table 3 shows the median HCC in each diagnosis group. There was no significant difference of means between S1 and S2 levels in either group. There was no significant difference of mean log(HCC) across diagnostic groups. We performed a post-hoc Tukey test for differences in pair-wise combinations and found none.

Table 4 shows HCC in segment 1, grouped by prescription history for both lifetime prescriptions and in the 180 days preceding death. We found that individuals with a lifetime history of prescription with psychotropic medication had significantly higher HCC than individuals with no prescription. Looking at subgroups, the difference remained significant for prescriptions for benzodiazepines, for antidepressants in general, and for SSRI. For prescriptions in the 180 days preceding death, the results were similar.

We also performed linear regression with both length of lifetime admissions to psychiatric wards and history of at least one coerced admission as covariates, but found no association with HCC in either raw or adjusted models.

5. Discussion

We did not demonstrate an association between increased HCC and psychiatric diagnosis compared with controls, in accordance with some previous studies [20,21]. In a review on cortisol-level studies in schizophrenia patients, Bradley and Dinan concluded that mere association of HCC with psychiatric diagnosis may be an oversimplification of the matter, suggesting both chronicity (e.g. debut psychosis seems to be associated with higher basal cortisol, while chronic schizophrenia is more heterogeneous) and the effects of treatment also should be considered [24]. Similar points have been made regarding HCC and affective disorders, obsessive-compulsive disorder and post-traumatic stress disorder [19,23,36]. In a recent systematic review of the literature, Koumantarou et al. concluded that the variability in the results previously reported may indicate that the relationship between HPA-axis activity and mental illness is not a straightforward linear relationship, but it may also reflect differences in methodology in case group definition, such as subtyping of diagnosis group (e.g. primarily melancholic vs. psychotic depression), sampling and analysis methods (illness stage when sample was taken, immunoassay-based vs. spectroscopic methods), and various confounders (e.g. age, comorbidity, adverse events in childhood) [23].

It should be noted that even our control group consists of individuals referred for a forensic autopsy and originally suspected of having a mental illness based on police report information, meaning they cannot be considered healthy controls. While some of these cases may be erroneous due to witness testimony based on rumor, some will most probably be individuals who had either a psychiatric diagnosis proper or at least some psychiatric symptoms, but even so were never admitted to a hospital. In
Table 3
Median HCC and mean log(HCC) in each group. P-value from one-way ANOVA, adjusted p-values from linear model controlled for age and gender. BIP: bipolar disorder; DEP: depressive disorder; SCZ: schizophrenia; OTH: other psychiatric diagnosis; CON: control group (no psychiatric diagnosis according to registry data); S1: hair segment 1; S2: hair segment 2.

<table>
<thead>
<tr>
<th></th>
<th>BIP (n = 5)</th>
<th>DEP (n = 12)</th>
<th>SCZ (29)</th>
<th>OTH (30)</th>
<th>CON (31)</th>
<th>p-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1:</td>
<td>5.9</td>
<td>15</td>
<td>16</td>
<td>24</td>
<td>16</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>S1:</td>
<td>2.02 (1.0)</td>
<td>2.84 (1.0)</td>
<td>3.11 (1.5)</td>
<td>3.4 (1.4)</td>
<td>3.02 (1.4)</td>
<td>0.28</td>
<td>0.18</td>
</tr>
<tr>
<td>S2:</td>
<td>3.5</td>
<td>18</td>
<td>18.5</td>
<td>36</td>
<td>20</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>S2:</td>
<td>1.63 (1.4)</td>
<td>2.92 (1.0)</td>
<td>2.94 (1.4)</td>
<td>3.48 (1.6)</td>
<td>3.23 (1.7)</td>
<td>0.14</td>
<td>0.088</td>
</tr>
</tbody>
</table>

Table 3 Shows median HCC and mean log(HCC) with SD for each of the five diagnostic groups.

Table 4
Association between prescription history (upper rows show lifetime, lower rows the 180 days preceding death) various psychotropic drug types and log(HCC). Unadjusted p-values from one-way ANOVA on log(HCC), adjusted p-values from linear model controlled for age and gender. SSRI: selective serotonin reuptake inhibitor. Drug classes followed in parentheses by anatomical therapeutical chemical (ATC) classification codes used for registry lookup.

<table>
<thead>
<tr>
<th>Hair cortisol concentration (pg/mg)</th>
<th>Prescription</th>
<th>No prescription</th>
<th>p-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of prescription with any type of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any psychotropic medication</td>
<td>73.8</td>
<td>9.6</td>
<td>0.0042**</td>
<td>0.0025**</td>
</tr>
<tr>
<td>Antipsychotic (N05A*)</td>
<td>74.7</td>
<td>51.2</td>
<td>0.44</td>
<td>0.40</td>
</tr>
<tr>
<td>Antidepressant (N06A*)</td>
<td>82.8</td>
<td>36.0</td>
<td>0.013*</td>
<td>0.0078**</td>
</tr>
<tr>
<td>SSRI (N06AB*)</td>
<td>88.4</td>
<td>37.7</td>
<td>0.028*</td>
<td>0.018*</td>
</tr>
<tr>
<td>Benzodiazepines (N05BA*, N05CD* and N05CF*)</td>
<td>79.8</td>
<td>25.6</td>
<td>0.0062**</td>
<td>0.0059**</td>
</tr>
<tr>
<td>Prescriptions in the 180 days before death with any type of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any psychotropic medication</td>
<td>73.5</td>
<td>51.2</td>
<td>0.064</td>
<td>0.047*</td>
</tr>
<tr>
<td>Antipsychotic (N05A*)</td>
<td>71.4</td>
<td>63.7</td>
<td>0.47</td>
<td>0.44</td>
</tr>
<tr>
<td>Antidepressant (N06A*)</td>
<td>79.6</td>
<td>59.1</td>
<td>0.17</td>
<td>0.13</td>
</tr>
<tr>
<td>SSRI (N06AB*)</td>
<td>119.5</td>
<td>55.2</td>
<td>0.0063**</td>
<td>0.0035**</td>
</tr>
<tr>
<td>Benzodiazepines (N05BA*, N05CD* and N05CF*)</td>
<td>99.8</td>
<td>43.4</td>
<td>0.0010**</td>
<td>0.0058**</td>
</tr>
</tbody>
</table>

Denmark, most cases of mild to moderate depression, anxiety, etc. are handled entirely by the general practitioners, who do not report diagnosis data to the National Patient Registry. In a previous study on healthy, adult volunteers, using the same analysis method as described in this paper, we found HCC in the range of 1.7–9.1 pg/mg [31]. This could suggest HCC is generally increased in the SURVIVE population, which could be due to other factors than psychiatric diagnosis. Regarding validity of the registry-based diagnoses, previous studies have shown the accuracy for the diagnoses schizophrenia and severe depressive disorder to be high, 97.5 % and 82.8 %, respectively [37,38]. We did not find an association between HCC and neither total length of lifetime admissions nor a history of coerced admission, which could both be considered proxy measures for mental illness severity. Only two decedents had been coercively admitted in the 180 days preceding death, both had near-normal levels of HCC (range: 9.7–38 pg/mg). Twenty-seven decedents (25.2 % of total population) had been admitted at least once to a psychiatric ward in the 180 days preceding death, and there was no significant difference of mean log(HCC) in these cases compared to cases who had not been admitted in the 180 days prior to death (difference -0.31 to 0.73, p = 0.4161). In order to perform a more thorough analysis, we would have preferred data on specific symptomatology and illness severity, e.g. psychometric scoring results, but unfortunately such data was not available.

We found that higher HCC was associated with a prescription history for both SSRI antidepressants, antidepressants as a whole, and benzodiazepines across all diagnosis groups, but not associated with prescription history for antipsychotics. The association was true for both lifetime prescriptions and prescriptions only expedited in the 180 days before death, suggesting it may both be the treatment itself but also the indication, i.e. the psychopathology that is associated with increased HCC. Our results indicated that studies evaluating HCC as a proxy for stress should include medication history to account for masking by treatment.

We have previously in internal analyses found that external cortisol contamination from cortisone-containing hand cream from one-self or family members may occur [31]. Such medications are available over-the-counter in Denmark and it was thus not possible to control for use of cortisol creams in the months preceding death. However, we did obtain prescription data regarding medication with cortisol content, including prescription cortisol creams. We found no significant difference of mean log(HCC) in patients with a prescription for corticosteroid containing medicine, either for life-time prescriptions or prescriptions in the 180 days preceding death, compared with decedents who were not prescribed such medication.

In conclusion, our study did not find an association between HCC and a registry-validated diagnosis of severe mental illness. We found significantly higher HCC in individuals with a history of prescription for psychotropic medication, especially benzodiazepines and SSRI. Number of days of admission and a history of coerced admission was not associated with higher HCC. Hair sampling is a novel matrix for measuring HPA-axis biomarkers such as cortisol, especially in the context of autopsy based research, but standardized analysis methods and reference ranges for healthy adult HCC should be introduced in order to improve interpretation and comparison between studies.

Declaration of Competing Interests
The authors have no conflicts of interest to declare.

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