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Postmortem Urinary Cortisol Levels in Relation to the Cause of Death

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Abstract

In 239 postmortem cases, we used liquid chromatography/time-of-flight mass spectrometry to measure urinary cortisol concentrations standardized to creatinine, which were then related to causes of death. For comparison, we also analyzed urine samples from 95 living subjects implicated in criminal cases. The median of the postmortem group (18.8 µmol cortisol/mol creatinine) was not significantly different from that of the living group (18.1 µmol cortisol/mol creatinine), but the highest value in the postmortem group was more than ten times higher than that in the living group (3730 versus 354 µmol cortisol/mol creatinine). Among the postmortem cases, 28% had values exceeding the previously reported upper 95% range for normal living subjects (52.8 µmol cortisol/mol creatinine), as did 12%of the living group. The postmortem cases were divided into subgroups according to cause of death:asphyxiation, blunt force trauma, brain hemorrhage, cardiac-related death, drowning, fire-related death, gun or knife trauma, infection, internal bleeding, ketoacidosis, miscellaneous and poisoning. The median cortisol levels of the ketoacidosisand the infection subgroups (respectively, 152 and 243 µmol cortisol/mol creatinine) were significantly higher than the median of the other subgroups (15.5 µmol cortisol/mol creatinine); however, the spread of data does not allow for any conclusions on an individual case level.

Keywords: Cortisol; Cause of death; Liquid chromatography/time-of-flight mass spectrometry; Postmortem; Urine biochemistry

Introduction

Except for in sudden deaths, the death process is likely to coincide with some degree of stress related to psychological and/or physical conditions, such as anxiety, pain, troubled breathing, and multiorgan failure [1]. Thus, stress marker measurement may be of interest in cases where the circumstances relating to death are unclear. Several studiesof postmortem cases have reported the urine or blood levels of the stress markers adrenaline and noradrenaline, or their metabolites [2-5]. The results have been somewhat unclear, but the postmortem levels have generally been higher than in living subjects. Some researchers have concluded that the elevated postmortem levels of catecholamines in blood and urine are an expression of tissue release, e.g., postmortem redistribution [6]. Others have judged that these levels relate to various causes of death [3,5].

Several investigations have reported the postmortem measurements of catecholamine, while only few studies have examined postmortem levels of the stress marker cortisol. Finlayson et al. [7] studied 15 infants and 20 adults who all died suddenly or accidently, and found that the cortisol concentrations in their postmortem blood did not significantly differ from the reference levels from living persons. However, Erkut et al. [1,8] found very high postmortem cortisol levels in the serum and in cerebrospinal fluid of patients with Alzheimer's disease or multiple sclerosis; they concluded that these levels were of similar magnitude to those observed in critically ill patients, such as those with septic shock or massive bleeding. Additionally, there are several published case reports relating to adrenocortical diseases, showing low postmortem serum or urine cortisol levels measured in cases with adrenal insufficiency [9-11].

Apart from investigations of the relation to typical adrenocortical diseases and some CNS diseases, the possible relationship between various causes of death and urinary cortisol levels has not yet been systematically evaluated. Being a stress marker, cortisol might be an indicator that can distinguish between a prolonged stressful course to death and shorter less stressful death processes, which could be valuable in a forensic context, where medical history and information about the circumstances of death are often limited [12-14]. The present study aimed to evaluate whether postmortem urine cortisol levels might be related to the cause of death.

Materials and Methods

This study included all postmortem cases during an approximately half-year period, for which urine could be obtained and a toxicological analysis was requested. No cases, where treatment with steroids was reported, were included. In total, 239 cases were analyzed, including 194 males and 45 females, aged from 18-98 years with a median of 48 years. In each case, autopsy was performed within 1–4 days after the bodies arrived at the forensic pathology department, and bodies were stored at 5°C until autopsy. For comparison, we also analyzed urine from 95 living persons, including 40 males and 55 females, aged from 12-74 with a median of 25 years; these subjects had blood and urine samples taken for toxicological analysis as part of criminal investigations (violence and rape).

The postmortem cases were subdivided by cause of death into the following subgroups: asphyxiation, blunt force trauma, brain hemorrhage, cardiac-related death, drowning, fire-related death, gun or knife trauma, infection, internal bleeding, ketoacidosis, poisoning, and miscellaneous. The miscellaneous subgroup included deaths of unknown cause, and causes of death observed for only one person. This grouping follows that of Zhu et al. [3] with slight modifications. Ketoacidosis as cause of death was defined as cases where the summed

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concentration of acetone, acetoacetate, and beta-hydroxybutyrate in blood was above 2000 µmol/kg and where diabetes or alcoholism was present and no other plausible cause of death was found.

Urine samples were analyzed for cortisol as recently described by Lang et al. [15]. Briefly, 0.25 mL urine was diluted with 0.75 mL water, followed by extraction using a fully automated 96-well solid phase extraction system. Cortisol and the internal standard cortisol-d3 were quantified using a Waters ACQUITY UHPLC system coupled to a Waters SYNAPT G2 time-of-flight mass spectrometer. The CV% ranged from 8–10%, and the accuracy of the method was >90%. The cortisol results were normalized in relation to the creatinine concentration in urine measured by a Reflotron Plus analyzer (Roche Diagnostics Systems, Basel, Switzerland).

Nonparametric statistical methods were used for descriptions of data and for testing group differences (Mann-Whitney for two groups and Kruskall-Wallis for multiple groups). Dunnett's test was used for post-hoc comparisons on log-transformed data.

Results

Urinary cortisol levels ranged from 0.4-3730 µmol/mol creatinine, with a median of 18.8µmol/mol for the 239 postmortem cases. For the living subjects, the range extended from 1.9–354 µmol/mol with a median of 18.1 µmol/mol. Both distributions were skewed to the right with a few high values (Figure 1). The medians were not significantly different (Mann-Whitney test); however, the upper limit of cortisol values in dead subjects was over ten times higher than that of living persons. There were no significant differences between the genders of the groups. The ten postmortem cases with the highest cortisol values (477-3730 µmol/mol) comprised four cases of poisoning (two of which were combined with pneumonia), one diabetic ketoacidosis case, two infection cases, one brain lesion case, one drowning, and one case with an unclear cause of death that was probably acetaminophen poisoning associated with some degree of ketoacidosis.

Figure 2 shows the relationship between urinary cortisol levels and cause of death. In general, there were large overlaps between the subgroups, and the medians ranged from 4.1-243 µmol cortisol/mol (Table 1). The Kruskall-Wallis test showed a significant overall difference between the subgroups ($p< 0.0001$), and the medians of three subgroups clearly deviated from the total group median of 18.6 µmol cortisol/mol: fire casualty (4.1 µmol cortisol/mol), ketoacidosis (152 µmol cortisol/mol), and infection (244 µmol cortisol/mol). Dunnett's test on log-transformed values showed that the cortisol levels of the ketoacidosis and infection subgroups were significantly higher than the rest of the values (both $p<0.05$), whereas the values of the fire casualty subgroup were not significantly different from the other subgroups ($p>0.05$). The infection subgroup consisted of five cases with pneumonia and one with tuberculosis. In the ketoacidosis subgroup, five cases were related to alcoholism and nine cases were of the diabetic type. The poisoning subgroup was the largest including 83 cases, 62 of which were caused by methadone, morphine, or a combination of several drugs. Although the median cortisol level in the poisoning subgroup (14.2 µmol/mol) was close to the overall median value, this subgroup included the lowest and the highest observed overall values; this variation probably reflects the heterogeneity of this subgroup, comprising cases with both rapid and delayed time courses up to death. The subgroup of cardiac-related deaths was similarly heterogeneous. Dividing the subgroup into acute versus non-acute cardiac-related deaths, showed 18 acute cases with a median of 13.8 and range of 1.6-387 µmol cortisol/mol, and 27 non-acute cases with a median of 34.0 and range of 1.3-182 µmol cortisol/mol. However, the medians were not significantly different. There were no cases of suspected adrenocortical diseases.

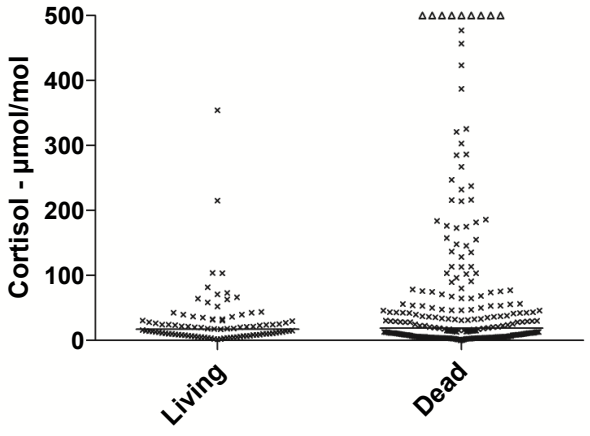

Figure 1: Cortisol levels of living persons versus dead persons. Truncated at 500 µmol/mol

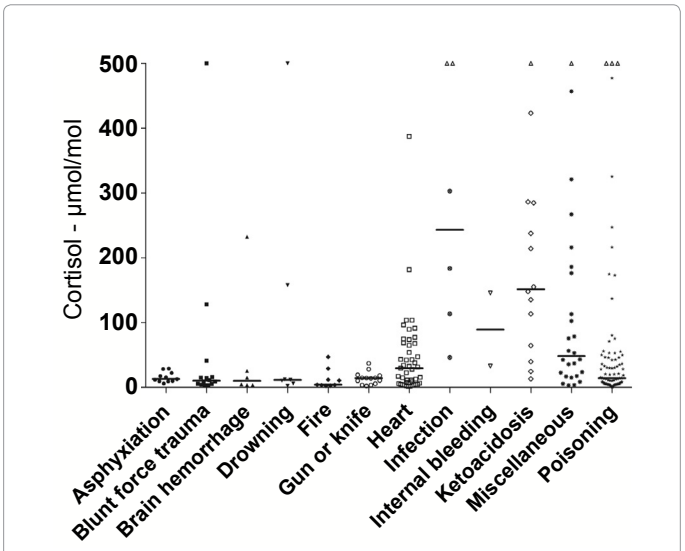

Figure 2: Cortisol levels according to cause of death. The horizontal bars show the median values. Truncated at 500 µmol /mol.

Cause of death	N	Mean	Median	Min	Max
Asphyxiation	11	15.8	13	6.1	29.3
Bleeding	2	89.4	89.4	33.1	146
Blunt force trauma	14	68	10.4	3.4	684
Brain hemorrhage	6	47.7	10.1	3.8	232
Drowning	7	121	11.8	2.6	646
Fire	9	13	4.1	2.5	47.3
Gun or knife trauma	14	14.2	14.4	2.4	37.2
Heart	45	46.1	29.8	1.3	387
Infection	6	449	243	46.6	1118
Ketoacidosis	14	208	152	13.2	770
Miscellaneous	26	145	48.4	3.3	1382
Poisoning	85	122	14.4	0.4	3730

Table 1: Urinary cortisol values for the postmortem subgroups. The values are given as µmol/ mol creatinine.

Discussion

Cortisol is the major glucocorticoid mediating the body's stress reaction to physical and psychological strains; this stress response is generally regarded as a helpful survival mechanism. Cortisol secretion can increase within minutes in acute stress situations, and can stay at high levels for long periods, days or months, in chronic disease conditions. Cortisol levels in blood, serum, and urine are heavily increased (10-20 times) in critically ill patients, e.g., those with sepsis or other life-threatening diseases [16,17]. Thus, cortisol production increases in stressful conditions leading to death, which is reflected as increased urinary excretion of cortisol, and high urinary cortisol concentration is expected in many postmortem cases.

Here, we found that cortisol levels varied widely in the postmortem group, with a maximum value of 3730 µmol/mol. A previous study reported morning cortisol levels for 119 normal living subjects to be from 2.3 to 52.8 µmol/mol (95%-range), with a geometric mean of 10.9 [15]. Later in the day and in the evening, the levels were reported to decrease, such that the maximum evening value observed in healthy subjects was 20.8 µmol/mol [18]. In the present study, 28% (67/239) of the postmortem cases showed urinary cortisol values exceeding the normal range, and the maximum value was about forty times higher than that observed in healthy, living subjects. Furthermore, the upper part of the observed range corresponded to increases observed in seriously ill patients. In the group of living subjects in the present study, the values extended up to only about a tenth of the maximum value in the postmortem group, probably reflecting that none of the living subjects were critically ill. However, these values tended to be higher than those previously observed for normal subjects, with 12% 11/95) exceeding the upper reference range value of 52.8 µmol cortisol/mol reported by Hansen et al. [18]. Since the living subjects in the present study were implicated in criminal cases, either as offenders or victims, a relatively high level of a stress marker like cortisol may not be unexpected.

The main purpose of the present study was to investigate whether the measured urinary cortisol value could suggest certain types of deaths, which might be of value in investigating unclear postmortem cases. The high median values observed in the infection and ketoacidosis subgroups were in accordance with the relatively long and physically stressful course leading to death in these types of cases. Lower levels might be expected in cases with a presumably more rapid course e.g., asphyxiation and fire-related deaths as was also revealed as a trend in the present data. The poisoning subgroup displayed a very wide range of values, possibly reflecting the spectrum from rapid to delayed intoxication deaths. We also observed a trend towards higher values in chronic versus acute cardiac-related deaths. However, the wide dispersions in these subgroups make it difficult to draw conclusions in individual cases.

Measurement of catecholamine has also been used frequently to assess stress responses, and several studies have reported catecholamine concentrations in blood, serum, or urine of postmortem cases [2-5,19,20]. In general, the concentrations in blood or serum are much higher (by a hundred times or more) than those measured in living controls. Some authors have concluded that the values are related to the type of death process, while others conclude that the high values are mainly an expression of postmortem redistribution [2,3,5,20]. Catecholamine are present in the nerve endings diffusely dispersed in tissues, which make postmortem release to blood likely, supporting the latter conclusion. Results have been rather disconcordant for the various studies, e.g., concerning the significance of the ratio between adrenaline and noradrenaline concentrations, which has been proposed as a marker for hypothermal deaths [19,21]. Regarding measurements in urine, Tormey et al. [4] found elevated levels of adrenaline and noradrenaline in 75-80% of unselected autopsy cases compared to the levels in living subjects; they concluded that this increase was related to physiological stress in the time up to death. This pattern is somewhat similar to that observed for cortisol in the present study. Compared to catecholamines, the turn-over rate of cortisol is slower, making it a marker for the presence of stress over more prolonged periods.

In a postmortem context, there has been only limited interest in measuring cortisol. Finlayson et al. [7] did not observe a significant difference between blood cortisol values for living and dead persons; however, they only explored cases of sudden death in which the stress response may have been absent or limited. Swaab et al. [22] investigated postmortem cortisol levels in the cerebrospinal fluid of Alzheimer patients and control cases, and found levels in both groups to be about 20-fold higher than those in living subjects. Erkut et al. [8] found a similar relationship for cortisol in postmortem cerebrospinal fluid of multiple sclerosis patients, and concluded that the elevation probably related to the stressful event of dying since the relative elevation corresponded to the increase of cortisol observed in blood or serum of critically ill patients. In a further postmortem study of Alzheimer patients, Erkut et al. [1] investigated whether psychological or physiological stress was likely to be most important with regard to death-related stress; they found that the most severely demented patients actually displayed the highest stress response and that administration of morphine did not have any impact. Thus, it was concluded that physiological rather than psychological stress in the dying period was decisive for the cortisol stress response.

Conclusions

The median urinary cortisol level for the total postmortem group was not significantly different from that of living persons subject to criminal investigation; however, the postmortem values displayed a wider range, with a maximum value exceeding that of the living group by more than a factor of ten. There were significant differences between subgroups with different causes of death; ketoacidosis and infection cases displayed increased values compared to other subgroups. These types of cases are likely to involve a long period of agony, which may be associated with increased levels of the stress marker cortisol. The spread of data did not allow for any conclusions on an individual case level.

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