Post¬-Mortem Serum level of Cortisol and its Relation with the Cause, the Time, and circumstances of Death: A Cross-sectional Cadaveric Study

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Abstract

Introduction: Cortisol is one of the stress markers in addition to catecholamine and other chemicals. **Aim of the study**: to measure the postmortem level of cortisol in people who died after various traumatic injuries to find its relationship with the date of death, cause of death, and circumstances surrounding death **Patients and methods:** This is a cross-sectional study included 50 cadavers, 33 males and 17 females (mean age 32.28 years). Samples of blood were collected and submitted to chemical analysis to measure the level of serum cortisol. ELISA. Was applied, which is based on the competitive interaction of cortisol and the hormone; the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of cortisol in the specimen, a blue color develops changing to yellow after stopping the reaction, the intensity of the color is inversely proportional to the amount of cortisol in the specimen. The absorbance was measured by spectrophotometry at 450 nm wavelength as soon as possible or within 30 min. Another sample of 50 living patients was included as a control for the level of cortisol. **Results:** The study showed that there is statistically significant mean difference (p-value = 0.0002) in cortisol levels between live and dead persons. There were no statistically significant differences in cortisol levels between males and females, as well as various age groups. The highest level of cortisol was recorded in patients who died suddenly followed by a gunshot. The highest level was recorded after 24 hours. The highest level was recorded in patients who died due to chest injury followed by head injury. **Conclusion:** Cortisol level in blood is significantly higher in dead persons compared to its level in living; this might be explained by the stressful agonal period before death, Cortisol levels did not show a positive correlation with time after death, however, it showed abrupt rise after the first 12 hours after death which continue after 38hours, This may be explained by the beginning of decomposition. There is a strong correlation between the postmortem level of cortisol and the cause of death; being high in sudden death, and firearm injuries, Cortisol levels are significantly higher in chest injuries compared to other regions of the body. **Recommendation**: Based on the study, the following recommendations are suggested: Ongoing research about post-to-mortem serum level of Cortisol Causes and circumstances of death.

Keywords: Traumatic injuries, Serum cortisol, Postmortem, Cause of death, Time of death, Site of injuries.

Introduction

The estimation of time since death and the determination of the cause of death are the two most challenging issues in the field of forensic sciences. In the literature, there is a lot of work that have been done to resolve these two major problems, and many parameters, biological, physical, chemical, and biochemical have been adopted to answer questions regarding how and when a person dies, especially in cases with legal aspects **(Meurs et al.,2019).** Several investigations have been carried out on post-mortem measurements of stress markers such as catecholamine **(Barry-Heffernan et al., 2019)** while only a few studies have been done on the measurement of post-mortem levels of cortisol as a stress marker except for sudden death **(Bonilla et al., 2018).** In deaths occurring after severe trauma, some degree of mental and/or physical conditions are associated with stress, such as anxiety, pain, troubled breathing, and multi-organ failure. Thus, the stress marker measurement may be of interest in cases where the circumstances relating to death are unclear.

Cortisol, the stress hormone is a steroid hormone synthesized and released by the cortex of the suprarenal gland. It belongs to the glucocorticoid groups of adrenal hormones that increase the sugar contents of the blood **(Papadimitriou and Priftis, 2009).** Cortisol also helps in keeping normal blood pressure and, at higher levels, it increases the blood pressure. Cortisol is a very active hormone and can be detected in all parts of the body. The level of cortisol shows variations during 24 hours (circadian rhythm); it reaches its highest level in the morning and drops in the afternoon **(Thau et al.,2024).** The secretion of cortisol from the adrenal gland is under the control of the hypothalamus through the hypothalamic-pituitary-adrenal axis (HPA). The corticotropin-releasing factor (CRH), which is secreted by the hypothalamus stimulates cells in the adjacent anterior lobe of the pituitary gland to secrete another hormone; the adrenocorticotropic hormone (ACTH) into the bloodstream, and then to the adrenal cortex. ACTH stimulates the synthesis of cortisol and other glucocorticoids **(Jonathan et al., 2017).** The cortisol secretion is controlled by the nervous system. Cortisol levels increase dramatically in response to polytrauma and intra-abdominal contamination. **(Rex et al., 2020).**

Several studies about post-mortem levels of other stress markers have reported the urine or blood level of adrenaline and noradrenalin, or their metabolites. The results have been somewhat unclear, but post-mortem levels have generally been higher than in living subjects **(Clapper et al., 2008).** Some researchers concluded that high post-mortem levels of catecholamines in blood and urine are expressions of tissue release, e.g. postmortem redistribution. Others considered that these levels are related to various causes of death **(Wilke et al., 2007).**

This study aims to measure the postmortem level of cortisol, as a biochemical guide in deceased persons, and to find its relationship with the date of death and the cause of death. Thus, this study is conducted on cadavers who died after severe trauma to measure the level of Cortisol in the bloodstream after death as a possible marker to answer some questions regarding the cause of death, time of death, and circumstances surrounding death.

Samples and Method

This is a cross-sectional study that included 50 cadavers, 33 males and 17 females (mean age 32.28 yrs.); the study was conducted at the Institute of Forensic Medicine in Baghdad, where the study samples are collected from the cases of deaths after severe trauma. These cases were referred to the Institute for the period from 1/2/2019 to 1/5/2019. Blood samples were taken from the heart directly for each case using a disposable syringe, and then each sample was placed in a tube containing anticoagulant. In each sample, the serum was separated in the laboratory of the Institute with a centrifuge. Then, the serum was transferred to another test tube and stored until the completion of the sample collection of 50 cases. Information regarding the deceased, such as age, sex, cause of death date of death, and circumstances of death was taken from the autopsy request form, relatives, and forensic doctors.

ELISA. was applied, which is based on the competitive interaction of cortisol and the hormone; the amount of bound hormone-enzyme conjugate is inversely proportional to the concentration of cortisol in the specimen, a blue color develops changing to yellow after stopping the reaction, and the intensity of the color is inversely proportional to the amount of cortisol in the specimen. The absorbance was measured by spectrophotometry at 450 nm wavelength as soon as possible or within 30 min. Measured absorption was plotted against [CAL] concentrations in a line-line graph. Appropriate interpolation of plotted measuring points results in a calibration curve, from which the analytic concentration in the sample can be determined. For the calculation of analyst concentrations, we selected an appropriate and validated curve-fitting option (recommendation: point to point).

Statistical Analysis

Data entered and managed by using the SPSS V 0.25. Descriptive statistics (frequency table, percentage, and figures) and inferential statistics (Independent T-test, ANOVA, and Pearson Correlation Coefficient) were used. A P-value of < 0.05 is deemed statistically significant.

Results

A sample of 50 dead persons (mean age 32.28 yrs.) with different causes of mortality was investigated to assess cortisol levels in comparison with a group of live people (n=50) with a mean age of 32.25 yrs. Findings indicated a highly statistically significant mean difference (p-value = 0.0002) in cortisol levels between live and dead persons, figures (1,2 and 3).

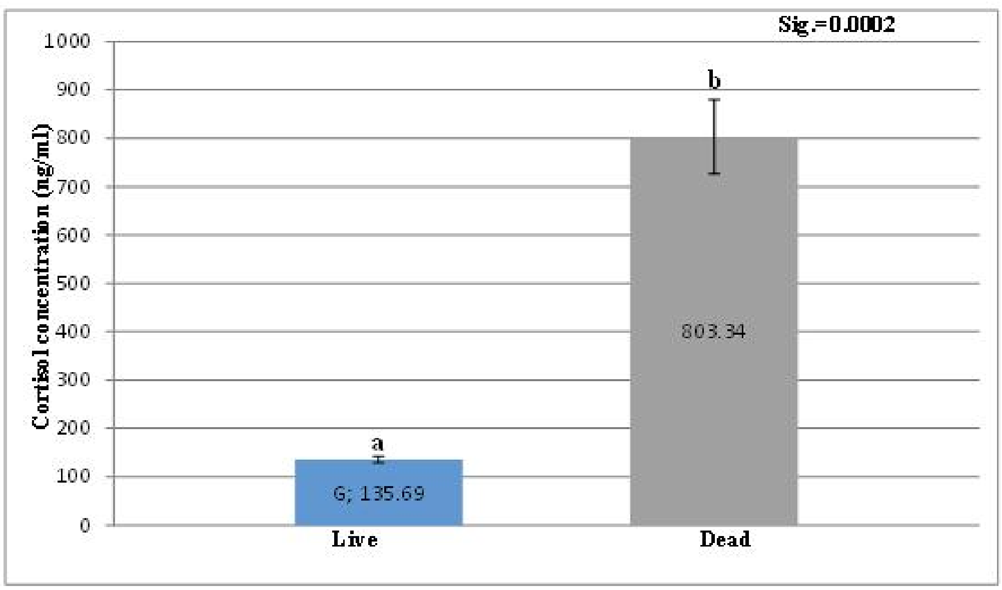

Figure 1: Displays mean levels of cortisol in live and dead persons, the difference between mean values is highly significant P=0.0002.

Table 1: Display distribution of mean Cortisol concentration according to Age group in (dead) male and female.

Age group (years)	Number	Mean ± SD
1 – 10	4	857.87±581.92 b
11 – 20	10	410.48±493.37 a
21 – 30	7	215.767± 401.06 a
31 – 40	12	415.30±407.1a b
41 – 50	11	432.3±386.45 a b
51 – 60	5	470.72±561.40 a b

Table 2: Display distribution of mean Cortisol concentrations according to Age group in (living).

Age group (years)	**Number of cases**	**Mean ± SD**
1 – 10	4	140.60± 54.78 abc
11 – 20	9	161.49 ±42.26 c
21 – 30	9	93 ±46.34 a
31 – 40	12	107.60 ±25.81 ab
41 – 50	10	160.39 ±59.816 c
51 – 60	6	156.17± 37.85 bc

Table 3: Displays concentration of Cortisol according to Time of Death

PMI (hours)	**Number of cases**	**Mean ±SD**
1 – 12	11	377.92± 495.10 a
13 – 24	24	433.08 ±453.73 a
41 – 50	10	160.39 ±59.816 c
51 – 60	6	156.17± 37.85 bc

Table 4: Displays the mean concentration of cortisol in a various cause of death (n=50).

Causes of death	N	Mean ± SD concentration of cortisol	Causes of death	P- value LSD
RTA	28	404.81 ±427.32	Gunshot	0.199
			Physical fighting	0.775
			Sudden death	0.294
			Blunt injury	0.020*
Gunshot	11	448.19± 468.68	RTA	0.176
			Physical fighting	0.257
			Sudden death	0.775
			Blunt injury	0.054
Physical fighting	3	131.05± 57.78	RTA	0.858
			Gunshot	0.257
			Sudden death	0.294
			Blunt injury	0.017*
Sudden death	5	968.34± 481.04	RTA	0.010*
			Gunshot	0.054
			Physical fighting	0.017*
			Blunt injury	0.020*
Blunt injury	3	68.96± 73.72	RTA	0.176
			Gunshot	0.858
			Physical fighting	0.199
			Sudden death	0.010*

* Significant differences, P≤ 0.05, M = Mean, S.D.=Standard deviation, N= Sample number

There are clear differences in mean cortisol concentration related to causes of death, Multiple comparisons with the ANOVA/ Duncan HSD test indicated that the highest mean value of cortisol concentration was seen in thoracic injuries in comparison with abdomen and extremities (p-value <0.05).

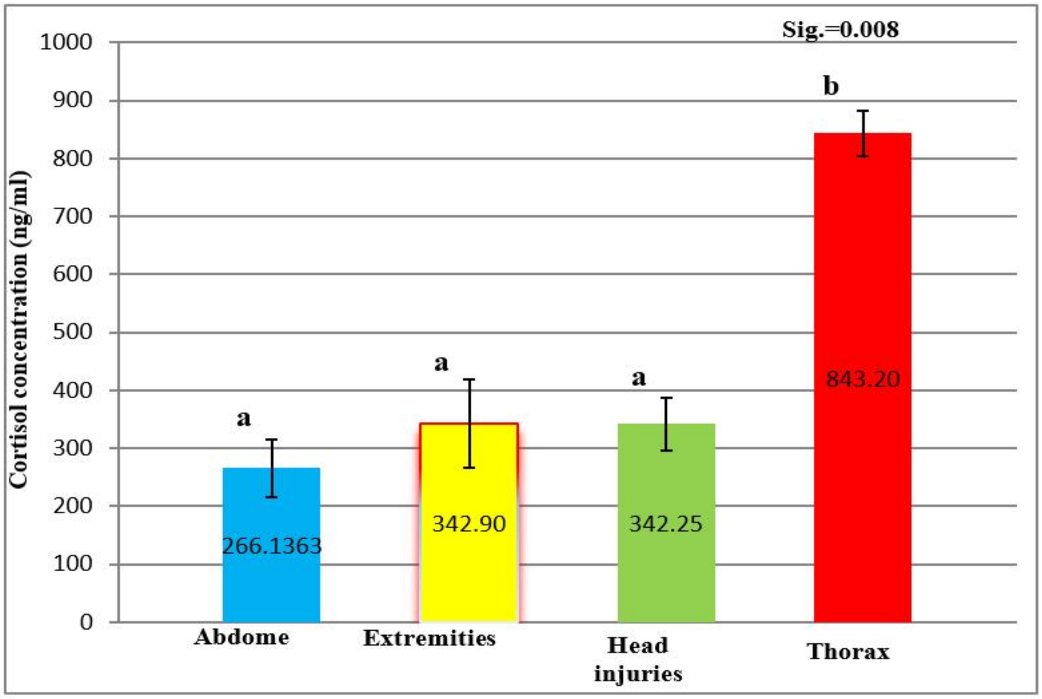

Figure2: Displays concentration of cortisol according to the site of trauma, b thorax

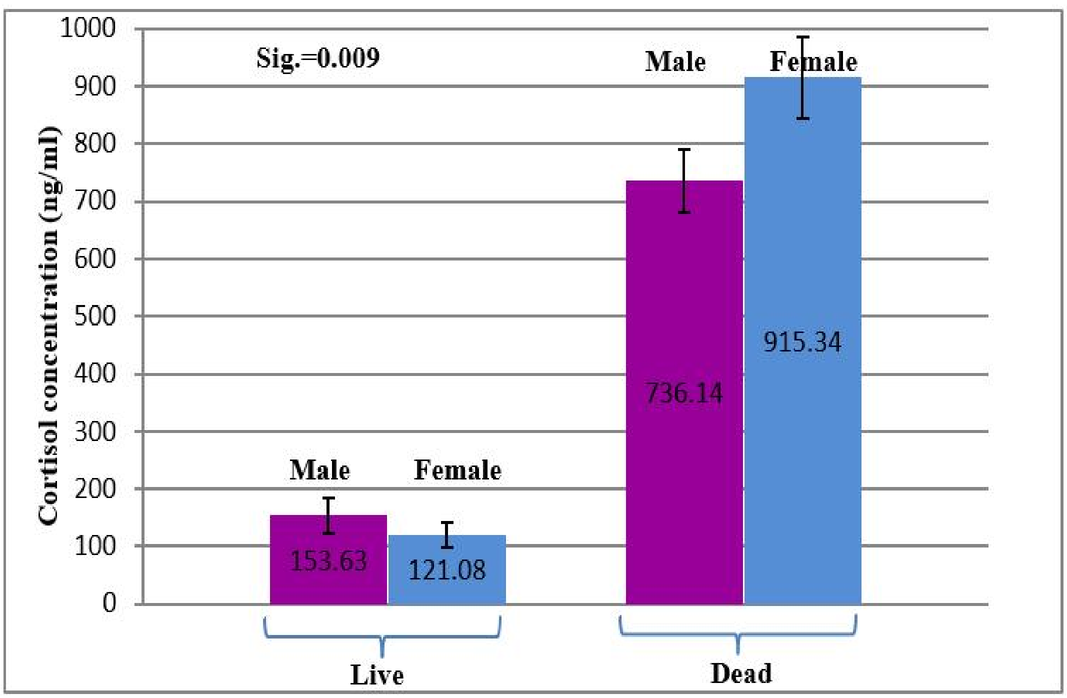

Figure 3: Display the concentration of cortisol in living and dead persons in males and females

The mean cortisol level in males vs. females was 380.91 ng/ml, and 517.51 ng/ ml respectively, with no significant difference in cortisol mean level between males and females (Independent t-test: t -934, def. 25, sig. 0359).

Discussion

Cortisol is an important hormone for life, especially when the human body is subject to stress such as major surgery; severe illness, or severe trauma. The measurement of cortisol levels in the bloodstream is indicated in clinical abnormalities related to glucocorticoid production **(Sekeris, 2002).** for instance, suspected Cushing's syndrome, and Addison's disease of adrenal insufficiency. In forensic medicine, many studies have been done on dead persons after different time intervals and on various causes of death, to find a relationship between the post-mortem level of cortisol in serum and the time of death and cause of death **(Matuszewski, 2017).**

Age and Gender

The present study showed (Table (4), and Figure (4)), that there are no statistically significant differences in cortisol levels between males and females, as well as various age groups. However, the mean cortisol concentration was higher in women (517.5ng/ml) than in men (380.9ng/ml). This study also showed that the average concentration of cortisol was high in the age group (1-10 years) (856.86ng/ml) and decreased in the age group (11-20 years) to (410.47ng/ml) and then down to (215.76ng/ml) in age group (21-20 years) then in age group (31-50 years) the concentration of cortisol rises to (432.29ng/ml) and in age group (51 -60 years) the concentration becomes (552.74 ng/ml). Examining the span of ages and trends in males and females separately where median cortisol was > 350 nmol/L, women had higher cortisol than men. On the other hand, in a study on the concentration of cortisol in CSF of brain ventricles, it has been found **(Wang et al., 2024).**

The level of cortisol in CSF of brain ventricles was significantly increased in old age and senile dementia, this means that the hypothalamo-pituitary-adrenal (HPA) axis is activated during aging and even more so in dementia. Accordingly, it was postulated that increased levels of corticosteroids may be neurotoxic. However, the CSF-cortisol levels in pre-senile and senile Alzheimer patients were similar. There was no significant correlation observed in the Alzheimer patients between the age of onset of the dementia and CSF cortisol levels, or duration of Alzheimer's disease and CSF cortisol levels **(Dronse et al.,2023).**

The results of this study have shown that the postmortem concentration of cortisol was greatly increased in deceased persons compared to ambulatory control (Table 4-1and Table 4-2) by nearly 6 folds, with the highest concentration of 165 ng/ml and the lowest level of 102 ng/ml in living persons, and highest concentration at 858 ng/ml and lowest concentration at 228 ng/ml in deceased. These findings indicate that the HPA axis activity during dying is greatly increased, which is reflected by the high level of cortisol in the serum of dead individuals compared to living. Many studies have reported up to 5 to 15-fold increased blood cortisol levels in critically ill patients, such as patients with septic shock or severe bleeding **(Ilias et al., 2023).**

It has also been reported that there was a positive correlation between the degree of elevation of endogenous cortisol levels and the survival rate in critical disease conditions, the strong increase in endogenous cortisol is presumed to be a protective reflex of the organism against a fatal threat **(Tenorio-Lopes & Kinkead, 2021).** The results of our study are in line with these studies as to the high post-mortem level of cortisol. Whether the rise in cortisol levels during dying was due to psychological or physical factors it was found that the stress of death which leads to extreme elevations in cortisol levels is of an organic (physical) nature rather than a psychological one as it is not suppressed by high-dose morphine administration **(Shayganfard, 2022).**

4.1 Postmortem Cortisol concentration and Time after Death

The results of the present study have shown that, though the levels of cortisol are higher in dead persons than in living they remain stable during the early stage of death; within the first 12 hours after death (table 4-3), but after that time the concentration increased suddenly and progressively with passage of time. This finding is in line with **(Anil, 2016).** other studies reported that the blood levels of cortisol remain stable during the early stage following death until 18 hours, after which it starts to rise progressively. This sudden and progressive rise in cortisol levels may be due to tissue decomposition which begins at 24 hours, under normal conditions and temperate climate. Tissue decomposition results in the release of its content cortisol to extracellular space thus, increased levels in the blood **(Nariai et al., 2022).** The current study has shown that there is a progressive increase in the level of cortisol with time, but there was no linear correlation between them table 3.

4.2 Cortisol's Concentration and Causes of Death

The present study has shown that the levels displayed a wider range of causes of death, with a maximum value exceeding that of the living group by more than a factor of six. There were no significant differences between subgroups with different causes of death; sudden death, gunshot, and RTA cases displayed increased values compared to other subgroups (table 4-4). These results are from other studies **(Gerritsen et al., 2019).** The explanation for these high levels of cortisol in these cases might be that these types of cases are likely to involve a long period of agony, which may be associated with increased levels of stress marker, which is cortisol. The present study also showed that there is no significant statistical correlation between mean cortisol levels and most causes of death. The RTA and gunshot cases (Independent T-test: t -0.278, def. 37, Sig. 0.783).

The highest mean value was seen in sudden death (968ng/ml) followed by gunshot injuries (448ng\ml), and then RTA injuries 404ng/ml, and the lowest level was seen in persons who died from fall from height (68ng/ml) (Table 4-4). Though the comparison between the mean level of cortisol in cases of sudden death and cases of fall from height revealed a highly significant difference, the possible relationship between various causes of death and cortisol levels has not yet been systematically evaluated. As a stress marker, cortisol might be an indicator that can distinguish between a prolonged stressful course to death, e.g. RTA, and shorter less stressful death processes, as in a fall from height **(Rex et al., 2020).** This issue could be valuable in a forensic context, where medical history and information about the circumstances of death are often limited, such as in cases of death in prisons after hard and prolonged physical torture **(Gerritsen et al., 2019).**

Clinical studies have been carried out on the estimation of the level of cortisol after head injuries and following surgery on the brain **(Kwon et al., 2019).** They have reported that the plasma cortisol level in the death sub-group was higher in comparison with the survival sub-group. However, plasma cortisol levels returned to normalcy after 7th day in the survival sub-group, in contrast to death sub-group patients where plasma cortisol levels persisted to be at higher concentrations. Cortisol levels were higher in patients with severe trauma than those with mild trauma in addition, the level of cortisol rises immediately after trauma or surgery and remains high during the first 12 hours then stabilizes and declines, and subsequently returns to normal **(Zada et al., 2013).** These observations may support our results which showed that cortisol levels in patients who died after head injuries were lower than those in the thorax, and extremities (figure 4).

Conclusion

The result of this study showed that there is a statistically significant difference in the mean concentration of cortisol in trauma involving the thorax compared to those involving the abdomen, head, and extremities. The explanation for this finding is that chest injuries, especially those associated with fractures of ribs and lung lacerations and contusions are usually associated with hypoxia difficulty of breathing, and even suffocation. This usually results in very stressful conditions and prolonged agonal period to death. Consequently, the adrenal gland will produce a larger amount of stress hormone in response to these stressful situations, and thus its level in blood increases. There are no previous postmortem studies on the level of cortisol at the site of traumatic injuries; this study is the first one.

Recommendation

Based on the study, the following recommendations are suggested:

- Ongoing research about post-to-mortem serum level of Cortisol Causes and circumstances of death.

Conflict of Interest

The authors declare no conflict of interest.

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