

**MORPHO METRIC ANALYSIS OF ADRENAL GLANDS IN ACCIDENT DEATH CASES AT
VARIOUS POSTMORTEM INTERVALS OF TIME.**

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ABSTRACT

INTRODUCTION: The adrenal glands are the endocrine in nature which secrete hormones usually called as freight, flight, fight hormones produced by the medulla of the gland. The cortex of the gland secretes the mineralocorticoids especially the Aldosterone that regulates the electrolyte balance and blood pressure, glucocorticoids that suppresses the immune system aids in metabolism.

Material and methods: 93 pairs of autopsy specimens were collected from the forensic department within an interval of 12-16, 16-20, 20-24hrs of death, and the specimen morphology was measured and analyzed statistically.

Results; there were differences in the right and left adrenal glands in relation to the length, breadth, thickness, and weight of the glands at various intervals of time after the death.

Discussion: There was not much difference in the morphometric parameters of the LAG and RAG

Conclusion: Considering the time of death and postmortem intervals there were changes which are to be noted with respect to the morphometric parameters of the Adrenal glands in sudden /Accidental death cases.

Keywords: Adrenal gland, time intervals, length, breadth weight, thickness

INTRODUCTION

The human adrenal glands are a pair of important life-saving endocrine glands situated over the upper pole of each kidney the right adrenal gland is pyramidal and left is crescentic in shape (Fig.1&2). Each gland is 5 cm in length (L), 3 cm in breadth (B), 1 cm in thickness (T), and 5 gm in weight (wt.)^{1, 2,3, 4}

The adrenal gland consists of the outer cortex and the inner medulla. Both parts of the gland differ from each other in structure, function, and development.



Fig.1 The Right adrenal gland



Fig.2 The Left adrenal gland

The outer zone of cortex ZG secretes mineralocorticoids predominantly aldosterone which act on distal convoluted tubules of the kidney to absorb water & sodium and excrete potassium to maintain the electrolyte balance in the body, which is called life-saving hormone. The middle zone of the cortex is ZF which secretes glucocorticoids predominantly cortisol which is the most important hormone to maintain the normal glucose level during stress conditions and initiates glucolysis, gluconeogenesis, and lipolysis to liberate energy during emergencies and is involved in various functions like increasing the blood pressure, immune response, vascular volumes and metabolisms of carbohydrate, protein, and lipids.⁵The inner zone of cortex ZR secretes sex steroids predominantly dehydroepiandrosterone (DHEA) which is responsible for secondary sexual characteristics.⁵

The physical trauma, heavy exercises, prolonged exposure to cold, pain, infection, shock, fright, and all emotional situations lead to stress and increasing cortisol levels in circulation. The release of adrenalin and activity of the sympathetic nervous system(SNS) increases the stress response. Chronic Psychological stress may cause immunosuppression.⁶

Road accidents have become alarming incidents in India due to heavy traffic and unconditional driving without proper safety precautions. The prevalence of death rate is drastically increased every year in India particularly the young men who are suffering more in the society.⁷ The hemorrhage in severe physical trauma cases leads to fatal adrenal shock.⁸ The adrenal glands are target structures in the hypothalamic-pituitary-adrenal (HPA) axis, and sympathetic-adrenal-medullary axis. These axes are mainly involved in

maintaining the homeostasis and stress system. The cortisol level is increased in blood circulation due to sudden fear and severe pain at the time of trauma and failure of long loop negative feedback mechanism leads to balance of the cortisol level by the HPA axis^{5,9,10}, and changes take place in dimensions, volumes, and histomorphological aspects of adrenal glands.^{5,9} The adrenal weight and medullary content are increased due to chronic stress during the depression, which also induces adrenal growth by cellular hypertrophy and hyperplasia. The increasing adrenal weight depends on increased size of the adrenal cortex.

The cells of the adrenals respond to stress by lipid depletion. The Cortex may appear homogeneous. The cells of ZF appear compact due to devoid of lipids. These changes in lipid-rich cells of ZF lead widening of ZR with extension out to ZG.¹⁰

This research study was carried out to find the structural differences of human adrenals in the accident death autopsy cases through morphometric analysis in different age groups within interval of time after death, the autopsy examination enables useful data for Forensic surgeons, pathologists, histopathologists, Endocrinologist, radiologists, Surgeons, Medico-legal fraternity, and entire Medical faculty to determine the structural changes of adrenal glands in accident death cases

MATERIALS AND METHODS

Materials

Study Design: Descriptive Cross-sectional study

Study population: The 93 autopsy cases belonged to road accident deaths. The Sample size was determined based on a histomorphometric Study of the Proportion of Different Zones of the Adrenal Cortex-A Postmortem Study Authored by Dilruba S. The adrenal glands were collected during the postmortem examination and analysis done according to postmortem timings in between 12-24 hours after death.

Study setting (Site): PES Institute of Medical Sciences and Research, Kuppam, Chittoor district, Andhra Pradesh.

Study Duration: October 2018 to November 2019

SAMPLE SIZE CALCULATION - 93

The sample size was determined based on the Study Authored by Dilruba Siddiqua Description¹⁴ Histomorphometric Study of the Proportion of Different Zones of Adrenal Cortex- A Postmortem
n1=7.72± 0.48 (Mean of group A) n2=7.51±0.43 (Mean of Group B)

Formula: Based on the above formula the sample size required per group is 73. Considering a 10% loss to follow-up, the estimated total sample size required is 93 per group.

where,

$\delta = |\mu_2 - \mu_1|$ = absolute difference between two means $\sigma_1, \sigma_2 =$

variance of mean #1 and #2

n1 = sample size for group #1 n2 = sample

size for group #2

α = probability of type I error (usually 0.05) β = probability of type II error (usually 0.8) z = critical Z value for a given α or β

$Z_{\alpha/2}$: This depends on the level of significance, for 5% this is 1.96

Z_{β} : This depends on power, for 80% this is 0.84

Assumption	
Mean difference between both the groups (δ)	=0.21
Standard deviation for both the groups (σ)	=0.455
α (two-sided) = 0.05; β =0.80	
Formula	
$n = \frac{2\{Z_{1-\alpha/2} + Z_{1-\beta/2}\}^2 \times \sigma^2}{\delta^2}$	
Calculation	
$n = \frac{2\{1.96 + 0.84\}^2 \times 0.455^2}{0.21^2} = 73$	

The total sample was divided into 3 groups (Table-1)

Groups	Post mortem Time interval	No of accident cases
Group -1	12-16hrs	43
Group -2	16-22hrs	25
Group -3	20-24hrs	25

Table:1 Distribution of cases with in a period of time after death

INCLUSION CRITERIA : Adrenal glands from human autopsy cases belong to accident death and both genders with 20-50 years age group

Postmortem interval of 12-24 hrs

Exclusion Criteria:

Decomposed autopsy cases, Pregnant women, Burns, Age less than 20 and more than 50 years

Commence of autopsy time less than 12 hrs and more than 24 hrs.

Sampling Technique

Sampling technique/ **Sampling procedure:** purposive sampling

Statistical Method

- ❖ The data will be entered into MS Excel 2007 version and further analyzed by using STATA14.1 Version.
- ❖ For descriptive analysis, the categorical variables will be analyzed by using percentages and the continuous variables will be analyzed by calculating mean \pm Standard Deviation.
- ❖ For inferential analysis, the numerical data were analyzed using 't test, "p" <0.05 considered statistically significant.

Study tools

Fig.3. The dissection kit



Fig.4. Digital weighing machine



Fig.5. Vernier Calipers



Study Tools

The usual dissection kit (Fig.2). was used to clean and dissect the adrenal specimens to remove the fat and fascia.

The digital weighing machine was used to check the weight of the adrenal glands(Fig.4).

The length, breadth, and thickness of collected adrenals were measured by using a vernier caliper(Fig.5).

Samsung J7 prime 2, Android mobile 13megapixal camera was used to take the photographs when measuring the glands.

The numbering system maintained to the collected specimens

The post-mortem numbers were entered on jars containing specimens for identity and to correlate with history for photographs.

METHODOLOGY

The institutional human ethical clearance obtained from PES Institute of Medical Sciences and Research kuppam. The human adrenal glands were collected from the deceased belongs to road accident cases that underwent compulsory postmortem examination. The samples were collected with the help of forensic surgeons with due formalities, procedures, precautions, prior permission from the head of the institution, and with consent of immediate relatives of the deceased at Government Hospital Kuppam.

The collected adrenal gland specimens were stored in 10% formalin immediately to prevent autolysis, to reduce shrinkage and distortion. The surrounding entire fat was removed by meticulous dissection. The proper history of the deceased such as name, age, sex, place of death, time, and cause of death were collected from the Department of Forensic Medicine and Toxicology with the consent of kin and kin of the deceased.

Written informed consent was obtained from the immediate relatives of the deceased in English and the local language (Telugu)

The glands were taken from 10% formalin after 12-24hours of fixation to measure the length, breadth, and thickness of the right and left adrenal glands with an vernier caliper, and the weight of the glands was checked with the digital weighing machine (Fig.4) with accuracy of 0.1mg(ShimadzuAY220 Analytical balancer

Fig.6.Anterior view of right and left adrenal glands Fig.7. posterior view of right and left adrenal glands



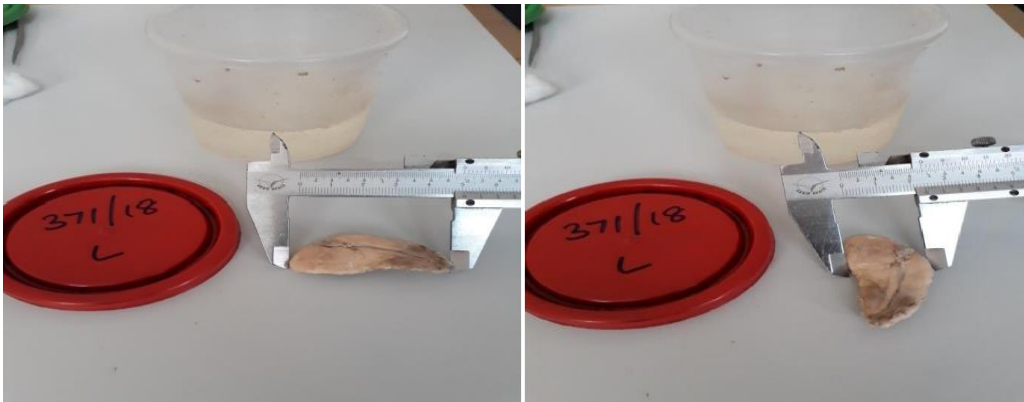
The lengths of both the right and left adrenal glands were measured at the base of the gland (Fig-11), the part which is related to the kidneys. The breadth of the right adrenal gland was measured from apex to base (Fig.11). The breadth of the left adrenal gland was measured at the level of the middle of the gland (Fig-9). The thickness of the glands was measured at the level of the hilum of both glands to maintain the uniformity where the supra renal vein is coming out of the glands. The weight was checked with a digital weighing machine with an accuracy of 0.1mg (Fig-8,10).

MORPHOMETRIC MEASUREMENTS OF ADRENAL GLANDS

Fig.8. Measurement of Left Adrenal Gland Thickness and Weight



Fig 9..Measurement of Left Adrenal Gland Length and Breadth



MEASUREMENT OF RIGHT ADRENAL GLAND

Fig.10.Measurement of Thickness and Weight



Fig.11.Measurement of Length and Breadth



RESULTS:

At 12-16 hours' duration after the death the length of right adrenal gland is less than the left whereas the breadth and weight of the right gland is more than the left and statistical significance was shown for the length and breadth of the glands

Table:2 12-16 hours' duration after the death

Variable	No of cases	Mean± S.D	P-Value	T-test
Rt Length	43	3.86±0.5	0.00	-8.3
Lt Length	43	4.55±0.4		
Rt Breadth	43	2.13±0.4	0.00	3.6
Lt Breadth	43	2.04±0.3		
Rt Thickness	43	0.90±0.2	0.11	-1.5
Lt Thickness	43	0.97±0.2		

Rt Weight	43	3.75±0.9	0.28	-1.08
Lt Weight	43	3.90±0.9		

Table:3 16-20 hours' duration after the death

Variable	No of cases	Mean± S.D	P-Value	T-test
Rt Length	25	3.67±0.4	0.00	-6.5
Lt Length	25	4.34±0.4		
Rt Breadth	25	2.3±0.4	0.00	5.6
Lt Breadth	25	1.79±0.3		
Rt Thickness	25	0.84±0.1	0.14	-1.5
Lt Thickness	25	0.91±0.2		
Rt Weight	25	3.63±0.7	0.48	0.70
Lt Weight	25	3.54±0.8		

At this interval of time (16-20hrs) the right gland breadth and weight are more than the left gland whereas the left gland length and thickness are more than the right adrenal gland. statistical significance was shown for the length and breadth of the glands

Table:4 20-24 hours' duration after the death

Variable	No of cases	Mean± S.D	P-Value	T-test
Rt Length	25	3.98±0.6	0.00	-6.8
Lt Length	25	4.57±0.5		
Rt Breadth	25	2.28±0.4	0.00	4.7
Lt Breadth	25	1.89±0.3		
Rt Thickness	25	0.94±0.2	0.37	-0.9
Lt Thickness	25	0.99±0.2		
Rt Weight	25	3.83±0.9	0.59	0.54
Lt Weight	25	3.75±0.8		

At 20-24 hours' duration after the death length and thickness of the left adrenal gland are more than the right adrenal gland whereas weight and breadth are more for the right adrenal gland than the left one. statistical significance was shown for the length and breadth of the glands. Comparison was done using T-test but there was no significance was noted statistically at various time intervals and among the parameters.

In the present study, the mean wt of RAG and LAG in 93 accident cases are 3.744g and 3.767g.

DISCUSSION

Weight of adrenal glands

William PL et al¹ 2000, Datta AK² 2005, Ranganathan TS³ 2006 and Krishna Garg¹¹ 2019 stated in their textbooks of human anatomy that each adrenal is 5gm weight in the present study it was comparatively less in the sudden death which was ranging between 3.744g -3.767g.

Lam KY et al¹² 2001 studied on adrenal glands collected from 333 cadavers in china and found the mean combined weight of adrenal glands is 11.8 gm which is heavier compare with results of present study and existing literatures. The author also reported that the LAG was heavier than the RAG and particularly heavier in young males.

Singh D et al¹³ 2004 conducted autopsy examination on 2025 cadavers in Chandigarh and reported that adrenal glands with a maximum weight at 40-50 years was 9.7 gm (right adrenal gland) and 11.3 gm (left adrenal gland) which corresponds with lam KY et al¹² but not in coherence with present results.

In a study reported by Annamraju et al¹⁵ 2016 the mean weight of adrenals in their study observed around 3.8gm with resemblance of results of present study which is not as much as reported in the writing by Lam et.al¹²2001· Singh D et.al¹³ 2004· Folligan et.al¹⁶ 2005 and is near the esteem revealed by weight from Vinay kumar et al¹⁷ 2004.

Anand MK et al¹⁸ 1998 did a study on 40 adrenals and found the mean weight of the glands were 4.28 gm. Hall and Hall¹⁹ 2021 explained that each adrenal gland weighs 4 gm, which is higher than weight of adrenals from accident

Narongchai duo²⁰ 2008 performed autopsy examination on 499 Thai cadavers and stated that the mean weight of the right adrenals in men and women were 5 gm and 7.9 gm and in the left adrenals of men and women are 6 gm and 8 gm respectively.

The present study reveals the fact that there is no difference in weight between the RAG & LAG and the findings of mean weight of the glands are consistent with the previous studies except that of Lam et al¹², Singh et al.¹³, and Narongchai duo²⁰, where the values were found higher.

In the present study the mean combined weight of human adrenal glands in 93 accident or sudden death autopsy cases is 7.5g, this results corroborates with a study done by Dorovini Zis K et al²¹ 1987 and the value is little higher in the study done by Dumser T et al²²1998 where the value is 9.3g and more higher which is 11g in male 15.9g in females in a study done by Narongchai duo et al²⁰ 2008 among Thailand population.

The mean combined weight of human adrenal glands in 93 suicide death autopsy cases is 10 g in the present study which is nearly equal to Dumser T et al²² 1998 where the value is 9.3g due to the cortical hypertrophy and depressive disorders.

According to Dilruba S et al¹⁴ 2010 the mean combined value of adrenal is 9.5g in human autopsy cases belongs to 11 to 60 years within 24-36 hours post mortem interval, which resembles the mean value (6-10g) mentioned by Sujith k Choudhary²³ 2011 and the value is less which is 8g by Harsh Mohan²⁴ 2021, Anil banshal²⁵ (6-8g) 2008 comparing with higher value 6-11g mentioned by Wlebke arlt²⁶ 2018 in their concern text books.

MORPHOMETRY (Length, breadth, Thickness)

Datta AK² 2005, Ranganathan TS³ 2006, Priya Ranganath²⁷ 2008, Sudha Seshayyan²⁸ 2016, Krishna Garg¹¹ 2019, Vishram Singh²⁹ 2021, and Subhadra Devi V³⁰ 2022 stated the mean value of L, B, T and wt of human adrenal glands are 5cm, 3cm, 1cm and 5g respectively mentioned in their literature of human anatomy and data deprived from human cadavers.

The mean length, breadth, thickness and weight are 3.7cm, 2.8cm, 0.6 cm and 3.8 g in the study done by Annam raju et al¹⁵ 2016, these findings are nearly in corroboration with results of the present study.

Lam et al¹² 2001 probably based on their studies on maximum number of sample size with adrenal glands in 333 autopsied Chinese bodies (208 men, 125 women) reported a mean longitudinal length of left adrenals (5.2cms) is more in the right adrenals (4.8 cm) due to difference in physical factors of population.

The results of few previous morphometric studies correlates with the dimensions in accidental death cases.

The author found no difference in dimensions and weight between the right side and left side adrenal glands and these results are conflict with present study results due to wide variations between RAG and LAG.

CONCLUSION: The morphometric parameters of the Adrenal glands changed mildly with different intervals of death and their duration of autopsy, which provides information for future researchers.

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