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RESEARCH ARTICLE

Sequential Postmortem Histological Changes In Human Adrenal Gland Up To Thirteen Hour & Thirty Minutes Post Mortem Interval.

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Abstract

This study was performed in Department of Anatomy in close association with the Department of Forensic Medicine & Toxicology, Pt.J.N.M.Medical Collage and Dr. B.R.Ambedkar Memorial Hospital Raipur (C.G.).Present study was done on human cadavers. Material for the present study was adrenal gland, taken directly from the dead bodies during postmortem examination. Human adrenal gland was obtained from cadavers at the time of autopsy. It was removed from cadavers with a known time of death. Death had resulted from trauma. The stages for which it was available were temperature between12.2/28.5-30.5/39.9^{0}C,humidity between 17/53 to 76/92% and duration range was between 6hrs to 13hrs30 min.In the present study 11 cases were studied. In each case adrenal gland was studied histologically. The adrenal cortex and medulla were studied. In this study increase in the rate of postmortem histological changes were found to be increased with rise in the temperature and duration.In the present study earliest remarkable sequential postmortem histological changes were seen in adrenal medulla i,e.cellular outline was not clear in most of the places and most of the nuclei were completely hyperchromatic at 6hrs PMI (Temp 12.2/28.5^{0}C). Post-mortem histological changes are directly dependent not only on the length of post-mortem time but also to a bigger extent on the temperature of environment. The rate of autolysis varies with environmental temperature, body size, nutritional status, pelage. The changes were found to be irregular in some cases. In the present study it was observed that sequential postmortem histological changes were different in some cases of same duration. The main reason lies in the fact that there are an extreme number of factors, which influence the post-mortem degradation of tissue in each case.. The rate of cellular degradation is increased by large carcass size, excessive adipose tissue, thick fur or wool and antemortem hyperthermia caused by pyrexia violent exercise or heat exhaustion.

INTRODUCTION

After death, a sequence of changes naturally occurs in the human body. Although these changes proceed in relatively order fashion, a variety of external factors and intrinsic characteristics may accelerate or retard decomposition. Understanding common postmortem changes and the variables that affect them allows the forensic

pathologist to more accurately estimate the postmortem interval and to provide a time frame during which death occurred.[1]

After death due to deprivation of blood supply, every organ undergoes series of gross as well as histological changes. Most of the organ in human body undergoes coagulative necrosis, cell swelling, cell membrane disruption, staining changes in cytoplasm, enzyme digestion of cellular organelles, nuclear changes like pyknosis, karyorrhexia and karyolysis.[2]

Autolysis represents the rotting of tissue without any vital reactions (without any signs of soreness). This phenomenon explained by Salkowaski(1890)as an intracellular enzyme activity and named "auto-digestion". Jacobi (1900) introduced the name "autolysis". It is a nonbacterial post-mortem self-destruction (decomposition) of tissue by its own enzymes[3,4,5] . It is a process that varies greatly from body to body, environment to environment, according to whether the body is clothed or naked, the circumstances of the death and the places where the body is found, the climate, and so forth[6,7,8].

Forensic pathologist throughout the world are trying to establish time passed since death by studying degenerative changes in organ and tissue at different intervals but definitive conclusion is still awaiting.

The histological studies on various tissues after death have been mostly confined to single organ or tissue by individual workers at different atmospheric conditions. Moreover very few workers work based on histological studies of postmortem tissue changes appears to have been undertaken by Indian and more so in Utter Pradesh. Since only a single organ was studied by most workers, any comparative evaluation of the varying rate of decomposition of the different organs and tissues cannot be made out.[9]

Although histological investigations plays an mechanism of death and injury, the postmortem autolytic process is dependent on various factors such as temperature, air humidity and the type of environment[2].

Sequential postmortem changes in adrenal gland have been reported in Porcine, Domesting Fowl and Pigs. Autolytic changes in the central nervous system, liver, gall bladder and lung have been studied.[10]

Adrenal gland changes - lipid depletion, fibrin thrombosis and necrosis. Each changes may occur an its own or in combination with another manifestation. In adult, the most frequently observed changes in that lipid depletion of the zona fasciculata of the cortex.[11] The intestines, suprarenal glands and spleen may putrefy in hours.[12]

In this study control cannot be taken because of random sampling. The samples are collected at the time of autopsy. Histological changes of tissue after death is influenced a great deal by atmospheric temperature and humidity besides other external and internal factors.

Till now the histological changes in adrenal gland after death have been studied in various animals, but yet very few studies with the same view have been done in human adrenal gland. That's why this present study is being carried out with this hope that it will be helpful to determine the sequence of postmortem histological changes in human adrenal gland.

MATERIAL AND METHOD

This study was performed in Department of Anatomy in close association with the Department of Forensic Medicine & Toxicology, Pt.J.N.M.Medical Collage and Dr. B.R.Ambedkar Memorial Hospital Raipur (C.G.).Present study was done on human cadavers. Material for the present study was adrenal gland, taken directly from the dead bodies during postmortem examination.

Human adrenal gland was obtained from cadavers at the time of autopsy. It was removed from cadavers with a known time of death. Death had resulted from trauma. The stages for which it was available were temperature between12.2/28.5-30.5/39.9^{0}C,humidity between 17/53 to 76/92% and duration range was between 6hrs to 13hrs30 min.

In the present study 11 cases were studied. In each case adrenal gland was studied histologically.

MATERIALS REQUIRED FOR THE STUDY

Materials required are:-

- Adrenal gland sample of 11 dead body.
- Plastic jars.
- 10% formalin.
- Scalpel, fine forceps, blunts forceps.
- Materials required for H & E stain.

INCLUSION CRITERIA

The selection of cases should be based on following criteria –

- Cases should be registered in Department of Forensic Medicine & Toxicology.
- Consent from Department of Forensic Medicine & Toxicology as well as from the attendant of dead individual will be taken before adrenal gland sample.
- Deceased without any history or evidence of any chronic disease.
- The exact time of death of individual should be known.
- All road traffic accidental cases are taken into account.

EXCLUSION CRITERIA

- Cases of unknown time of death.
- Disease Affecting Adrenal Gland i.e. Pheochromocytoma, Cushing's Syndrome, Addisons Disease, Pituitary Adenoma. Cases complicated by other metabolic disorders like Diabetic Mellitus, Hypertension.
- Burn and poison cases.
- Cases having pathology, affecting the cellular architecture or biochemical constituent of material.

Total 11 cases are of different age and sex was selected. The environmental temperature in ^{0}C (minimum/maximum) and humidity in % (minimum/maximum) was recorded from "India Meteorological Department, Meteorological Centre Raipur". After collection of adrenal gland from mortuary it was transported in 10% formalin solution for 48 hrs for fixation. Small pieces or block of adrenal gland tissues each 1- 2 cm thick were taken and H&E staining done for histological examination.

OBSERVATION

Sequential postmortem histological changes in human adrenal gland.

STUDY NO - 1

Postmortem interval (PMI) - 6hrs Temperature 12.2/28.5 ^{0}C, humidity 28/47%.

H & E staining

- Architecture is maintained.
- Cortex is easily differentiated from the medulla.

Zona glomerulosa:-

- epithelium is retracted from the basement membrane.
- most of the nuclei are completely hyperchromatic while some are hyperchromatic at periphery.
- pyknotic and vesicular nuclei are hardly seen.

Zona fasciculata:-

- sinusoids are slightly dilated.
- prominent endothelial cells are seen at few places.
- edematous changes in cells are seen at some of the places.
- most of the nuclei are completely hyperchromatic while vesicular and pyknotic nuclei are visible at few places.

Zona reticularis:-

- sinusoids are slightly dilated.
- most of the nuclei are hyperchromatic at periphery while some nuclei are completely hyperchromatic.
- vesicular nuclei are seen at some of the places.
- pyknotic nuclei are seen at few places.

Medulla:-

- cell outline is not visible in most of the places.
- karyolysis is seen in some of the places.
- most of the nuclei are completely hyperchromatic while some are

hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

STUDY NO - 2
PMI - 6hrs 10min, Temperature 26.4/41.1 ^{0}C, humidity 19/42%

H & E staining
- Architecture is somewhat maintained.
- Cortex can be differentiated from medulla.

Zona glomerulosa:-
- cellular outline is not clear.
- cytoplasm is vacuolated in most of the places.
- retraction of epithelium from the basement membrane.
- most of the nuclei are completely hyperchromatic while some nuclei are hyperchromatic at periphery and pyknotic.
- vesicular nuclei are hardly seen.

Zona fasciculata:-
- zonal architecture is disturbed.
- cellular outline is not clear.
- sinusoids are not visible.
- most of the nuclei are completely hyperchromatic and some are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

Zona reticularis:-
- cellular outline is not clear in most of the places.
- sinusoids are slightly dilated at few places.
- most of the nuclei are peripherally hyperchromatic while some nuclei are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

Medulla:-
- cellular outline is not clear.
- most of the nuclei are completely hyperchromatic while some nuclei are karyorrhectic.

STUDY NO - 3
PMI - 6hrs 50min Temperature 14.8/25.1 ^{0}C, humidity 30/59 %

H& E staining
- Architecture is maintained.
- Cortex is easily differentiated from the medulla.

Zona glomerulosa:-
- epithelium is retracted from the basement membrane.
- most of the nuclei are hyperchromatic at periphery.
- completely hyperchromatic nuclei are seen at some of the places.
- pyknotic nuclei are seen at few places while vesicular nuclei are hardly seen.

Zona fasciculata:-

- sinusoids are moderately dilated.
- prominent endothelial cells are seen at few places.
- most of the nuclei are hyperchromatic at periphery while completely hyperchromatic nuclei are seen at some of the places.
- pyknotic nuclei are seen at few places.

Zona reticularis:-

- cell outline is not clear at most of the places.
- sinusoids are moderately dilated.
- most of the nuclei are hyperchromatic at periphery while completely hyperchromatic nuclei are seen at few places.
- vesicular nuclei are seen at few of the places while pyknotic nuclei are hardly seen.

Medulla:-

- cellular outline is not clear in most of the places.
- complete lysis of cells at some of the places.
- most of the nuclei are completely hyperchromatic and some are hyperchromatic at periphery.
- pyknotic nuclei are seen at some of the places.

STUDY NO - 4

PMI- 8hrs Temperature 20.6/29.8^{0}C, humidity 65/83%

H & E staining

- Architecture is maintained.
- Cortex is easily differentiated from the medulla.

Zona glomerulosa:-

- retraction of epithelium from the basement membrane.
- most of the nuclei are hyperchromatic at periphery while some nuclei are completely hyperchromatic.
- pyknotic and vesicular nuclei are seen at few places.

Zona fasciculata:-

- zonal architecture is maintained.
- sinusoids are slightly dilated with few prominent endothelial cells.
- at some places cytoplasm is vacuolated.
- most of the nuclei are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

Zona reticularis:-

- sinusoids are slightly dilated.
- most of the nuclei are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

Medulla:-

- cellular outline is not clear.
- most of the nuclei are hyperchromatic at periphery and few nuclei are completely hyperchromatic.

STUDY NO - 5

PMI - 8hrs 10min Temperature 21.2/28.6^{0}C, humidity 47/77%

H & E staining

- Architecture is somewhat maintained.
- Cortex can be differentiated from the medulla.

Zona glomerulosa:-

- epithelium is retracted from the basement membrane.
- most of the nuclei are completely hyperchromatic while some nuclei are hyperchromatic at periphery.
- pyknotic and vesicular nuclei are hardly seen.
- cytoplasm is vacuolated at few places.

Zona fasciculata:-

- cells are edematous.
- zonal architecture is somewhat maintained.
- sinusoids are not seen.
- most of the nuclei are completely hyperchromatic while hyperchromasia at periphery is seen at few places.
- pyknotic nuclei are seen at very few places.

Zona reticularis:-

- cellular outline is not clear.
- sinusoids are not seen.
- most of the nuclei are completely hyperchromatic while some nuclei are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.
- karyorrhexis and karyolysis are seen at few places.

Medulla:-

- cellular outline is not clear.
- cytoplasm is vacuolated at some places.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at some of the places

STUDY NO - 6

PMI - 12hrs Temperature 29.1/37.8^{0}C, humidity 50/63%

H & E staining

- Architecture is maintained.
- Cortex is easily differentiated from medulla.

Zona glomerulosa:-

- retraction of epithelium from the basement membrane.
- most of the nuclei are completely hyperchromatic.
- hyperchromasia at periphery is seen in some of the places.
- pyknotic nuclei are hardly seen.

Zona fasciculata:-

- zonal architecture is maintained.
- retraction of epithelium from the basement membrane.
- sinusoids are moderately dilated with few prominent endothelial cells.
- most of the nuclei are completely hyperchromatic.
- some nuclei are hyperchromatic at periphery.
- pyknotic nuclei are hardly seen.

Zona reticularis:-

- sinusoids are moderately dilated with prominent endothelial cells.

- most of the nuclei are hyperchromatic at periphery.
- some nuclei are completely hyperchromatic and pyknotic.

Medulla:-

- cellular outline is not clear.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at some of the places.

STUDY NO - 7

PMI- 12hrs 10min Temperature 25.4/38.8^{0}C, humidity 25/57%

H & E staining

- Architecture is moderately disturbed.
- Cortex can be differentiated from the medulla.

Zona glomerulosa:-

- retraction of epithelium from the basement membrane.
- most of the nuclei are completely hyperchromatic while some nuclei are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

Zona fasciculata:-

- zonal architecture is slightly disturbed.
- sinusoids are slightly dilated.
- most of the nuclei are completely hyperchromatic while hyperchromasia at periphery is seen at some of the places.
- pyknotic nuclei are seen at few places.

Zona reticularis:-

- cells are shrunken.
- cellular outline is not clear.
- most of the nuclei are completely hyperchromatic and hyperchromasia at periphery is seen at few places.
- at few places karyorrhexis and karyolysis are also seen.

Medulla:-

- cellular outline is not clear.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at some of the places.
- karyorrhexis and karyolysis are seen at few places.

STUDY NO - 8

PMI - 12hrs 20min Temperature 24.9/39.4^{0}C, humidity 17/53%

H & E staining

- Architecture is moderately disturbed.
- Cortex can be differentiated from the medulla.

Zona glomerulosa:-

- retraction of epithelium from the basement membrane.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places.

Zona fasciculata:-

- zonal architecture is moderately disturbed.
- cells are edematous with vacuolated cytoplasm in most of the places.
- sinusoid are not well visualized.

- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places .

Zona reticularis:-
- cellular outline is not clear.
- sinusoids are moderately dilated.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places.

Medulla:-
- cellular outline is not clear.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places.

STUDY NO - 9
PMI-13hrs, Temperature 23.9/38.1^{0}c,humidity 18/50%
H & E staining:-
- Architecture is moderately disturbed.
- Cortex can be differentiated from the medulla.

Zona glomerulosa:-
- retraction of epithelium from the basement membrane.
- most of the nuclei are hyperchromatic at periphery and some nuclei are completely hyperchromatic.
- pyknotic nuclei are hardly visible.

Zona fasciculata:-
- zonal architecture is disturbed.
- cells are edematous.
- sinusoids are slightly dilated.
- most of the nuclei are hyperchromatic at periphery as well as completely hyperchromatic.
- pyknotic nuclei are hardly seen.

Zona reticularis:-
- cell outline is not clear.
- sinusoids are moderately dilated.
- most of the nuclei are hyperchromatic at periphery while some nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places.

Medulla:-
- cells are shrunken.
- most of the nuclei are completely hyperchromatic while some nuclei are pyknotic.

STUDY NO – 10
PMI - 13hrs 25min, Temperature 24.9/39.4^{0}C, humidity 17/53%
H & E staining
- Architecture is moderately disturbed.
- Cortex can be differentiated from the medulla.

Zona glomerulosa:-
- retraction of epithelium from the basement membrane.
- most of the nuclei are completely hyperchromatic.

Zona fasciculata:-
- zonal architecture is disturbed
- cells are edematous with vacuolated cytoplasm in most of the places.

- sinusoids are not visible.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places.
- karyolysis is seen at very few places.

Zona reticularis:-
- cellular outline is not clear.
- sinusoids are not visible.
- most of the nuclei are completely hyperchromatic.
- pyknotic nuclei are seen at few places.

Medulla:-
- cellular outline is not clear.
- most of the nuclei are completely hperchromatic.
- karyorrhectic and pyknotic nuclei are seen at few places.

STUDY NO- 11
PMI - 13hrs 30min Temperature 27.3/38.3^0 C , humidity 48/75%
H & E staining
- Architecture is somewhat maintained.
- Cortex is well differentiated from the medulla.

Zona glomerulosa:-
- retraction of epithelium from the basement membrane.
- most of the nuclei are hyperchromatic at periphery.
- completely hyperchromatic nuclei are seen at few places.
- pyknotic nuclei are hardly seen.

Zona fasciculata:-
- zonal architecture is maintained.
- sinusoids are slightly dilated with few prominent endothelial cells.
- cytoplasm is vacuolated in most of the places.
- most of the nuclei are hyperchromatic at periphery.
- some nuclei are completely hyperchromatic.
- pyknotic nuclei are hardly seen.

Zona reticularis:-
- sinusoids are moderately dilated with some prominent endothelial nuclei.
- most of the nuclei are completely hyperchromatic.
- some nuclei are hyperchromatic at periphery.
- pyknotic nuclei are seen at few places.

Medulla:-
- cellular outline is not clear.
- most of the nuclei are completely hyperchromatic as well as hyperchromatic at periphery.
- pyknotic and karyorrhectic nuclei are seen at few places.

DISCUSSION

After death a sequence of changes naturally occurs in the human body. Although these changes proceed in a relatively orderly fashion, a variety of external factors and intrinsic characteristics may accelerate or retard decomposition. Understanding common postmortem changes and the variables that affect them allow the forensic

pathologist to more accurately estimate the postmortem interval (PMI) and to provide a time fram during which death occurred.[1]

Autolysis is normally associated with autopsies and recognition of the phenomenon is very important[14,15], to elucidate forensic cases. Thus autolytic changes have been investigated by forensic pathologists, because they may assist in determining the time of death, especially in the first few hours.[11] Although postmortem autolysis depends on various factors, the most important factor is the postmortem period.[16,2]

It is known that after death or tissue/body removal, anoxic post-mortem effects (ischemia, glycolysis, and proteolysis) make changes in enzyme activity and ultra structural cells.[17,18] Autolytic process including changes in shape, size, electron density, and localization of cell structures and generally causes gradual loss of highly arranged structural organization of cells.[50] However, not only different types of tissue, but also different types of cells in a tissue or organ, as well as different organelles within one cell, can express different degree of sensitivity to autolytic process.[20]

Scarpelli et al established that occurrence of autolysis is quick in tissue, which have a high concentration of autolytic enzymes, such as pancreas and gastric mucosa, it is moderate in the heart, liver and kidney tissue, whereas it is slow in fibroblast, which are poor in lysosomes and hydrolytic enzymes.[52] Similar results were obtained by other authors who have studied the autolytic tissue changes.[50,53-56]. In present study sequential postmortem histological changes in human adrenal gland were observed in 30 cases of different age and sex. Average environmental temperature ranges between 12.9/28.5 - 30.5/39.9^{0}c, humidity between 17/53 - 76/92%.

Deborah Barber[10] found in porcine adrenal gland with the periodic acid schiff (PAS) reaction, the cells of the zona glomerulosa became shrunken, pyknotic and individualized within 6 hours after death at both 4^{0}c and 24^{0}c.Moderate to marked changes were noted in the zona reticularis by 12 hours at both temperatures. Medullary cells were moderately shrunken and hyperchromatic in refrigerated adrenal gland specimens through 96 hours, but at 24^{0}c, these changes were marked within 24 hours postmortem.

Hekmat Osman[21] observed in nicotine treated mice, the cells of the three cortical zones (zona fasciculata in particular) showed apparent increase in the cytoplasmic vacuolation , mitochondrial degeneration and increased lipid droplets. The nuclei showed abnormalities in the form of shrinkage, pyknosis and chromatin extension.

Smita patra et al[22] observed that the cells in zona glomerulosa showed increased nuclear density. Hypertrophy and hyperplasia of zona fasciculata with intracytoplasmic lipid depletion varying from mild to extensive, giving rise to parenchymal cord like arrangement of cells. Sinusoidal prominence in all the three zones including medulla.

Deborah Barber[10] found in porcine adrenal gland after 6 hours at 4^{0}c in zona glomerulosa, nuclear hyperchromasia was prominent where in zona fasciculata few nuclei were hyperchromatic. In zona reticularis there was decreased cytoplasmic stainability, cellular shrinkage and individualization proceeded at variable rates while in medulla shrinkage of cells was noted. After 6 hours at 24^{0}c in zona glomerulosa nuclei of this zone were markedly hyperchromatic, cells were shrunken and individualization was marked where in zona fasciculata cells of this zone have started to shrink and individualized, but the parallel cord architecture was intact. Chromatin clumping was visible within nuclei. Endothelial cells and sinusoids were easily seen. In zona reticularis sinusoids were markedly noticeable and cytoplasmic stainability decreased.

Leticia Rodrigues et al[11] observed in sublingual gland at 6 hrs postmortem, the nuclei presented karyorrhexis, irregular distribution of chromatin, which formed clumping along the nuclei periphery, and loss of limit.

Rajni Thakur[23] observed in kidney at 6 hrs PMI (18.5 /33.3^{0}c)- retraction of epithelium in PCT and DCT. In present study at 6 hrs PMI (12.2/ 28.5^{0}c) architecture was maintained and cortex was easily differentiated from medulla . In zona glomerulosa epithelium was retracted from the basement membrane and most of the nuclei were completely hyperchromatic while vesicular and pyknotic nuclei were hardly seen . In zona fasciculata sinusoids were slightly dilated with prominent endothelial cells, some cells were showed edematous changes . In zona reticularis, sinusoids were slightly dilated and most of the nuclei were hyperchromatic at periphery . In medulla cell outline was not clear in some places and most of the nuclei were completely hyperchromatic .

At 6 hrs 10min PMI (26.4/41.1^{0}c) architecture was somewhat maintained and cortex could be differentiated from medulla. In zona glomerulosa,retraction of epithelium was seen. Cytoplasm was vacuolated in most of the places. Most of the nuclei were completely hyperchromatic. In zona fasciculata zonal architecture was disturbed and sinusoids were not visible. In zona reticularis cell outline was not clear in most of the places and sinusoids were slightly dilated. In medulla most of the nuclei were completely hyperchromatic and karyorrhexis was first observed. At 6 hrs 50min PMI (14.8/25.1^{0}c) architecture was maintained and cortex was easily differentiated from medulla. In zona glomerulosa most of the nuclei were hyperchromatic at periphery. In zona fasciculata sinusoids were

moderately dilated with prominent endothelial cells and most of the nuclei were hyperchromatic at periphery while pyknotic nuclei were seen at few places. In zona reticularis cellular outline was not clear in most of the places and sinusoids were moderately dilated. Most of the nuclei were hyperchromatic at periphery. In medulla cellular outline was not clear. Karyolysis and pyknotic nuclei were seen at some of the places

Rajni Thakur [23] found at 8.30hrs PMI (9.8/26.3^{0}C) in PCT dark stained nuclei and vesicular nuclei were almost equal while in DCT number of vesicular nuclei was more than dark stained nuclei. In present study at 8hrs PMI (20.6/29.8^{0}C) architecture was maintained and cortex was easily differentiated from the medulla. In zona glomerulosa most of the nuclei were hyperchromatic at periphery while some nuclei were completely hyperchromatic . Pyknotic and vesicular nuclei were seen at few places. In zona fasciculata zonal architecture was maintained,sinusoids were slightly dilated and cytoplasm was vacuolated at some places. Most of the nuclei were hyperchromatic at periphery and pyknotic nuclei were seen at few places. In zona reticularis sinusoids were slightly dilated. Most of the nuclei were hyperchromatic at periphery while pyknotic nuclei were seen at few places. In medulla cellular outline was not clear and most of the nuclei were hyperchromatic at periphery and few were completely hyperchromatic.

At 8hrs 10min PMI (21.2/28.6^{0}C T) architecture was somewhat maintained and cortex could be differentiated from the medulla. In zona glomerulosa cytoplasm was vacuolated at few places. Most of the nuclei were completely hyperchromatic while some nuclei were hyperchromatic at periphery and pyknotic, vesicular nuclei were hardly seen. In zona fasciculata zonal architecture was somewhat maintained. Cells were edematous so that sinusoids were not well visualized. Most of the nuclei were completely hyperchromatic while hyperchromasia at periphery and pyknotic nuclei were seen at very few places. In zona reticularis cellular outline was not clear. Most of the nuclei were completely hyperchromatic while some were hyperchromatic at periphery, pyknotic nuclei were seen at few places. karyorrhexis and karyolysis were also seen at few places. In medulla cytoplasm was vacuolated at some places. Most of the nuclei were completely hyperchromatic and pyknotic nuclei were seen at some of the places.

Tomita Y et al[2] observed in wistar rat at 10hrs PMI(23^{0}c) edema in the proximal tubules, condensation of nuclear chromatin and edema in distal tubules and atrophy of acinar cells in the pancreas.

Leticia Rodrigues et al[11] observed in sublingual gland at 12hrs, almost all the acini presented intermediary stage of autolysis, despite the external limits remaining well defined. Some nuclei presented karyorrhexis and others were pyknotic and hyperchromatic due to high chromatin condensation.**Bryant BH et al**[12] observed in testis at 12hrs postmortem, clumping and margination of chromtin in leyding cells.**Kushwaha et al**[9] observed kidney in first 12hrs at temperature range 26-30^{0}c, there was mild cloudy swelling in cytoplasm, some cases show severe changes.**Rajni thakur**[23] found at 12.30hrs PMI(24.5/38.1^{0}C)in PCT retraction of epithelium with vesicular as well as darkly stained nuclei and edematous epithelial cells were also found.

Deborah Barber[10] found in porcine adrenal gland at 12hrs (4^{0}c) in zona fasciculata sinusoids were moderately prominent and endothelial cells were hyperchromatic. In zona reticularis sinusoids were prominent, cellular shrinkage and individualization being most pronounced. In medulla hyperchromatia was observed. At 12hrs (24^{0}c) in zona glomerulosa nearly all nuclei were pyknotic , cells were in the process of individualization. In zona fasciculata nuclei were markedly hyperchromatic and karyolysis was first noted. Disruption of the linear parallel cord architecture was moderate. In zona reticularis sinusoids prominence were decreased and pknotic nuclei were most noticeable. In medulla slight cellular shrinkage was noted. In present study at 12hrs (29.1/37.8^{0}C) architecture was maintained and cortex was easily differentiated from medulla. In zona glomerulosa most of the nuclei were completely hyperchromatic while hyperchromasia at periphery was seen in some places and pyknotic nuclei were hardly seen. In zona fasciculata zonal architecture was maintained and sinusoids were moderately dilated. Most of the nuclei were completely hyperchromatic while some nuclei were hyperchromatic at periphery. Pyknotic nuclei were hardly seen. In zona reticularis sinusoids were moderately dilated. Most of the nuclei were hyperchromatic at periphery while some nuclei were completely hyperchromatic and pyknotic. In medulla cellular outline was not clear, most of the nuclei were completely hyperchromatic and pyknotic nuclei were seen at some places. At 12hrs 10 min PMI (25.4/38.8^{0}C) architecture was moderately disturbed as compare to earlier hours and cortex could be differentiated from the medulla . In zona glomerulosa most of the nuclei were completely hyperchromatic while some nuclei were hyperchromatic at periphery. Pyknotic nuclei were seen at few places . In zona fasciculata zonal architecture was slightly disturbed and sinusoids were slightly dilated. Most of the nuclei were completely hyperchromatic and pyknotic nuclei were seen at few places . In zona reticularis cells were shrunken and outline was not clear. Most of the nuclei were completely hyperchromatic and at few places karyorrhexis and karyolysis were also seen . In medulla cellular outline was not clear. Most of the nuclei were completely hyperchromatic while karyorrhexis and karyolysis were also seen at few places .

At 12hrs 20min PMI (24.9/39.4^{0}C T) architecture was moderately disturbed and cortex could be differentiated from the medulla. In zona glomerulosa most of the nuclei were completely hyperchromatic and pyknotic nuclei were seen at few places. In zona fasciculata zonal architecture was moderately disturbed. Cells were edematous with vacuolated cytoplasm and sinusoids were not well visualized. Most of the nuclei were completely hyperchromatic and few were pyknotic. In zona reticularis cellular outline was not clear and sinusoids were moderately dilated. Most of the nuclei were completely hyperchromatic while few were pyknotic. In medulla cellular outline was not clear and rest finding were similar to zona reticularis.

Vinita kushwaha et al[9] found in their study after 13- 18 hrs PMI with increasing temperature of up to 31 to 35^{0}C, moderate& severe changes. Architecture maintained, more cloudy swelling and disruption of epithelium, glomeruli swollen. Only 2 out of 13 cases showed severe changes, architecture disrturbed, cloudy swelling and disruption of epithelium were prominent.

Shrikant verma[24] found in their study in human liver, the architecture of hepatic cord was maintained, blood was present in the central vein after 12.50 hrs (23.8 to 39.3^{0}C) and after 13 hrs PMI(8.2 to 23.9^{0}C).Sinusoids were studded with blood and perivascular space of Disse was also visible in deeper parts of sinusoids while in the subcapsular sinusoids perivascular space of Disse was not visible after 13 hrs PMI(8.2 to 23.9^{0}C).Blood was also present in the artery and vein of the portal triad after 13 hrs PMI(8.2 to 23.9^{0}C).

Rajni Thakur[23] found after 13 hrs at 23.9/38.7^{0}C,glomeruli were expanded and disrupted .At 25.5/31^{0}C, glomeruli were disrupted with presence of red blood cells while at 8.2/23.9^{0}C, glomeruli were expanded, disrupted with the presence of red blood cells. In all cases of 13 hrs PMI Bowman's spaces were increased with the presence of debris.Dark stained nuclei were seen in all cases.After 13 hrs (23.9/38.7^{0}C,) PCT were expanded with debris in the lumen.Retraction and disruption of the epithelium was observed.Epithelial cells were mostly anucleated. At places dark stained nuclei were seen but vesicular nuclei were hardly visible and in DCT debris in the lumen, disruption of epithelium with dark stained nuclei at few places were seen while epithelial cells were mostly anucleated. In CT retraction and disruption of epithelium with dark stained as well as vesicular nuclei and individualization of cells at places were observed. After 13 hrs PMI (23.9/38.7^{0}C)PCT expanded with disrupted epithelium having dark stained nuclei but at places anucleated also. Vesicular nuclei were rarely seen.DCT expanded with disrupted epithelium having vesicular to dark stained nuclei. CT at places with completely disrupted epithelium but also with maintained architecture at some places. Epithelial cells were seen with vesicular as well as dark stained nuclei while some of the epithelial cells were anucleated. After13hrsPMI (25.5/31^{0}C)PCT were expanded with disrupted epithelium having dark stained nuclei but anucleated epithelial cells were also present.DCT with disrupted epithelium having dark stained nuclei. CT were with retracted and disrupted epithelium along with vesicular as well as dark stained nuclei..In two cases of 13.10hrs PMI (27.3/42.2^{0}C,T) glomeruli were expanded with dark stained nuclei.PCT were expanded with retraction of epithelium at places in one case while retraction, disruption of epithelium in another case with vesicular and dark stained nuclei in the epithelium of PCT in both cases. DCT were seen with retraction and disruption of epithelium having dark stained nuclei as well as anucleated in both cases. CT were dilated with retraction and disruption of epithelium having vesicular nuclei mostly but anucleated epithelial cells were also present. Intestitium was visible in the cortex in both.After13 hrs PMI temp 23.9/38.7^{0}C and13.10hrs PMI(27.3/42.2^{0}C,T) visible intestitium was observed in the cortex.After13.30hrsPMI(29.6/43.1^{0}C, T) expanded glomeruli with vesicular to dark stained nuclei were seen.PCT were seen with retraction and disruption of epithelium having dark stained nuclei. Anucleated epithelial cells were also present.DCT with disruption of epithelium having vesicular nuclei were seen but anucleated epithelium cells were also present, CT with retraction and disruption of epithelium having dark stained nuclei were seen.

In present study at 13 hrs PMI (23.9/38.1^{0}c T) architecture was moderately disturbed and cortex could be differentiated from the medulla. In zona glomerulosa most of the nuclei were hyperchromatic at periphery and some were completely hyperchromatic while pyknotic nuclei were very few in number. In zona fasciculata zonal architecture was disturbed. Cells were edematous and sinusoids were slightly dilated. Most of the nuclei were hyperchromatic at periphery as well as completely hyperchromatic . Pyknotic nuclei were hardly seen.In Zona reticularis cell outline was not clear but sinusoids were moderately dilated. Most of the nuclei were hyperchromatic at periphery while some nuclei were completely hyperchromatic. Pyknotic nuclei were seen at few places .In medulla cells were shrunken and most of the nuclei were completely hyperchromatic while some nuclei were pyknotic . At 13hrs 25min PMI (24.9/39.4^{0}C) architecture was moderately disturbed and cortex could be differentiated from the medulla. In zona glomerulosa most of the nuclei were completely hyperchromatic. In zona fasciculata zonal architecture was disturbed and cells were edematous with vacuolated cytoplasm. Sinusoids were not visible. Most of the nuclei were completely hyperchromatic and karyolysis was seen at few places. In zona reticularis cellular outline was not clear and sinusoids were not visible most of the nuclei were completely

hyperchromatic while pyknotic nuclei were seen at few places. In medulla cellular outline was not clear. Most of the nuclei were completely hyperchromatic while karyorrhectic and pyknotic nuclei were seen at few places. At 13hrs 30min PMI (27.3/38.3^0 C) architecture was somewhat maintained and cortex was well differentiated from the medulla. In zona glomerulosa most of the nuclei were hyperchromatic at periphery while completely hyperchromatic nuclei were seen at few places and pyknotic nuclei were hardly seen. In Zona fasciculata zonal architecture was maintained and sinusoids were slightly dilated with few prominent endothelial cells, cytoplasm was vacuolated in some places. Most of the nuclei were hyperchromatic at periphery while some nuclei were completely hyperchromatic and pyknotic nuclei were hardly seen. In zona reticularis sinusoids were moderately dilated. Most of the nuclei were completely hyperchromatic and pyknotic nuclei were seen at few places . In medulla cellular outline was not clear. Most of the nuclei were completely hyperchromatic as well as hyperchromatic at periphery while pyknotic and karyorrhectic nuclei were seen at few places.

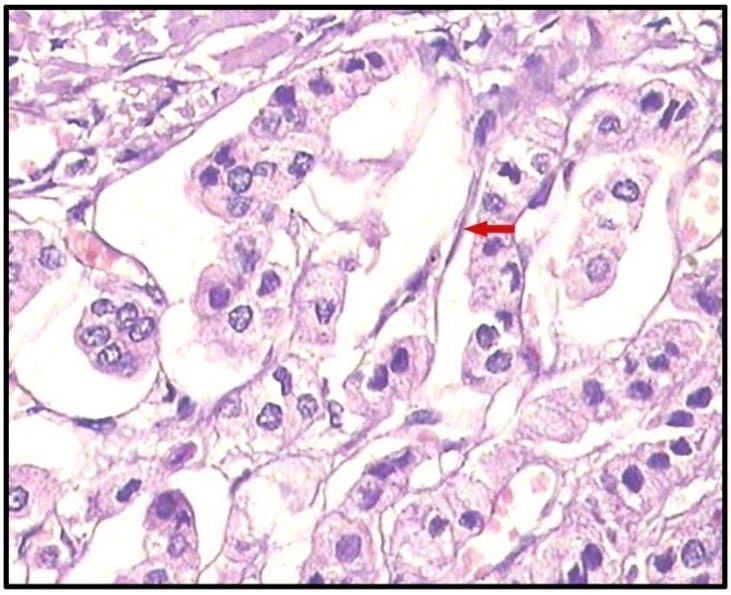

Fig.7: PMI - 6hrs Temp 12.9/28.5^{0}c,H&E stain 40x photomicrograph showing retraction of epithelium from the basement membrane in zona glomerulosa , most of the nuclei are completely dark stained with pyknotic changes at few places.

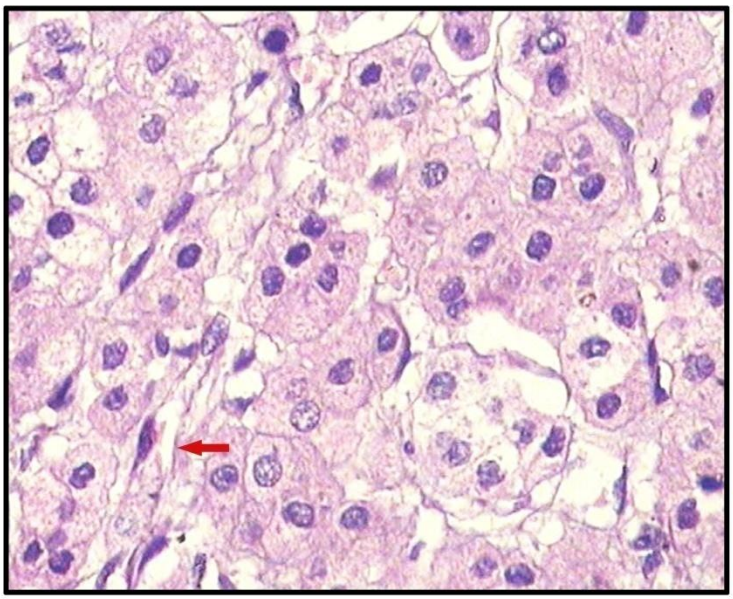

Fig. 8: PMI - 6hrs Temp 12.2/28.5^{0}c, H&E stain 40x ZF, photomicrograph showing sinusoids are slightly dilated , most of the nuclei are completely dark stained.

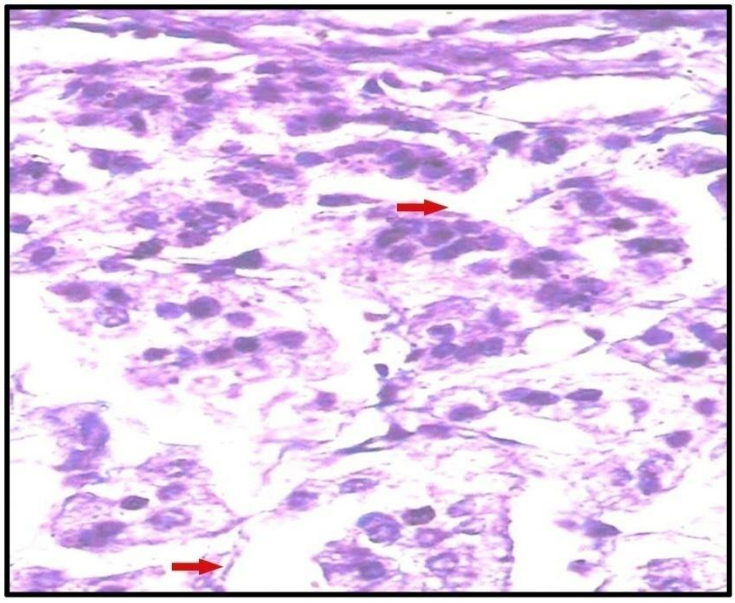

Fig. 13: PMI - 12hrs 10min Temp 25.4/38.8^{0}c,H&E stain 40x Zona glomerulosa; photomicrograph showing retraction of epithelium from the basement membrane, most of the nuclei are completely hyperchromatic, pyknotic nuclei are seen at few places.

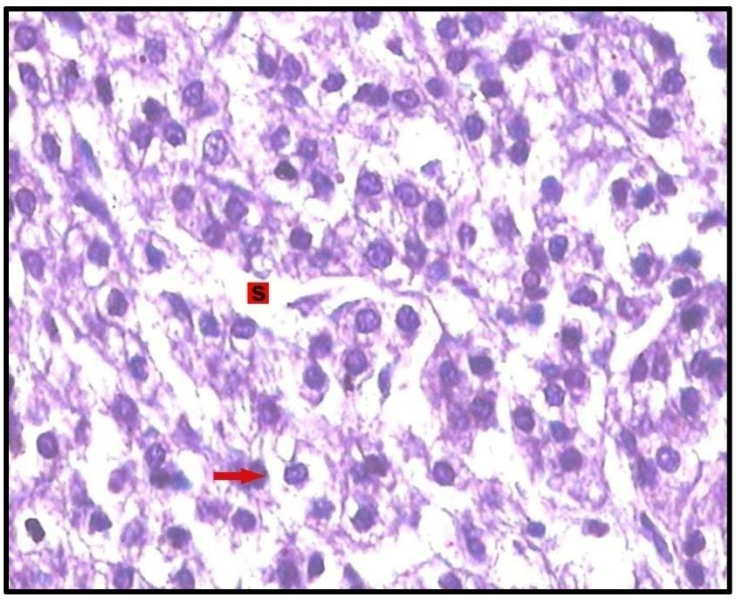

Fig. 14: PMI - 12hrs 10min Temp 25.4/38.8^{0}c, H&E stain 40x zona fasciculata; photomicrograph showing architecture is slightly disturbed, sinusoids are slightly dilated, most of the nuclei are completely hyperchromatic, pyknotic nuclei are seen at few places.

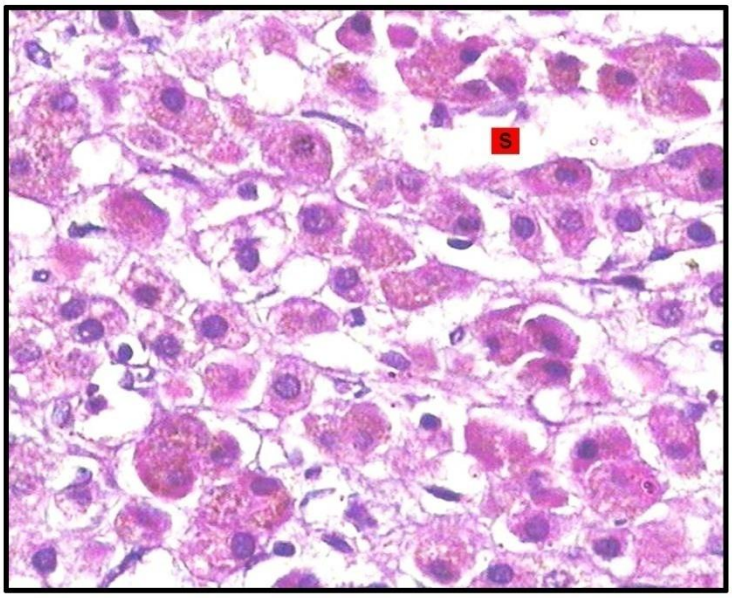

Fig. 17: PMI- 13hrs 30min Temp 27.3/38.3^{0}c H&E stain 40x Zona reticularis photomicrograph sinusoids are moderately dilated with prominent endothelial cells, most of the nuclei are completely hyperchromatic.

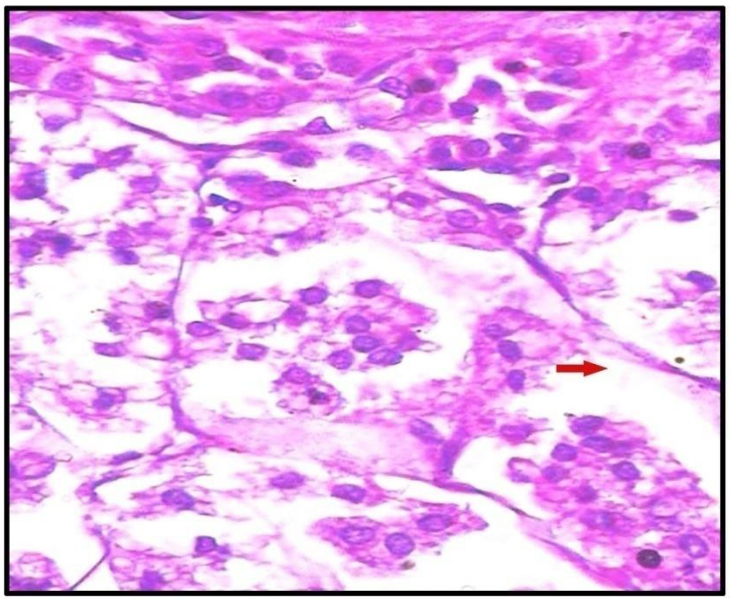

Fig. 18: PMI -15hrs Temp 20.2/31.6^{0}c H&E stain Zona glomerulosa photomicrograph showing retraction of epithelium from the basement membrane, cells are in the process of individualization.Most of the nuclei are completely hyperchromatic.

SUMMARY AND CONCLUSION

A study of sequential postmortem histological changes of adrenal gland was done in 11 random samples of human adrenal glands at different time intervals after death and in different temperature conditions. Adrenal glands were studied under the light microscope after staining with Harris haematoxyline and eosin in routine manner .

The adrenal cortex and medulla were studied. In this study increase in the rate of postmortem histological changes were found to be increased with rise in the temperature and duration.In the preasent study earliest remarkable sequential postmotem histological changes were seen in adrenal medulla i,e.cellular outline was not clear in most of the places and most of the nuclei were completely hyperchromatic at 6hrs PMI (Temp 12.2/28.5^{0}C).

Post-mortem histological changes are directly dependent not only on the length of post-mortem time but also to a bigger extent on the temperature of environment. The rate of autolysis varies with environmental temperature, body size, nutritional status, pelage.(**Deborah Barber[10]**)

The changes were found to be irregular in some cases. In the present study it was observed that sequential postmortem histological changes were different in some cases of same duration. The main reason lies in the fact that there are an extreme number of factors, which influence the post-mortem degradation of tissue in each case.(**Micozz MS[25]).** The rate of cellular degradation is increased by large carcass size, excessive adipose tissue, thick fur or wool and antemortem hyperthermia caused by pyrexia violent exercise or heat exhaustion.(Deborah Barber[10])

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