Histological Study of Adrenal Gland In Case Of Suicidal Deaths


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Abstract:

Aim & Objectives: To correlate the Histological adaptive changes in adrenal gland in response to chronic stress since it is a stress responding organ common to both the HPA axis & Sympathoadrenomedullary axis.

Material & Methods: Duration of study – Two yrs 
Place of study: Dept. of Anatomy, FMT, Pathology of MKCG MC, Berhampur & SCB MC, Cuttack.
Material for study – Right and Left adrenal.
No. of Suicidal cases -Hundred 
No. of Accidental cases (control)-Twenty .

Results: On Histological study it was found 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. A normal pattern of Adrenal gland is informative of receipt of sudden violence i.e accident.

Conclusion: The present study concludes and supports the idea that chronic stress as in suicide usually induces adrenal growth which may have implications for forensic people in revealing the cause of unknown deaths.

Key words: HPA axis, Stress, Suicide, Sympathoadrenomedullary axis.

I. Introduction

Uncertainty and instability are the norm of today’s work environment. Now-a-days suicidal cases account for more than a million deaths each year. The body’s reaction to a stressor is part of our survival mechanism. Under stress, the ACTH is released into circulation by the anterior pituitary. ACTH reaches the adrenal cortex where it stimulates the endocrine cells to secrete the steroid hormone cortisol. The Zona fasciculata of adrenal cortex is rich in lipids which acts as the raw material for the synthesis of cortisol. ACTH is known to have a proliferative effect on adrenal gland. It was demonstrated that its chronic liberation induces extracellular signal related kinases (ERKS), C-fos, C-jun, activin, spexine, adrenal NPY etc.to play a crucial role in the cellular proliferation induction. This work has been undertaken to study the histological adaptive changes in adrenal gland in such stressful conditions.

II. Materials and Methods :

 Duration of Study: 2 years ( Aug. 2010- July 2012 )
 Place of Study: Dept. of Anatomy – SCB MC, Cuttack
 Dept. of Pathology – SCB MC, Cuttack.
 Dept. of FMT – SCB MC.
 Material for study: Right & Left Adrenal.
 Number of suicidal cases: 100
 Number of control cases (accidental ): 20
 Method of Staining: Routine stain – H&E
 Special staining for lipids – OIL RED O

2.1 Inclusion Criteria:

All established cases of suicide with prominent autopsy findings and relevant history.
2.2 Exclusion Criteria:
- Cases with extremes of age groups i.e. < 15 yrs and > 60 yrs.
- Cases with doubtful and insufficient history.
- Suicidal cases which were brought for post mortem after 8hrs of death depending on the environmental temperature and cause of death.
- Pregnant ladies

1.3 Methods for Histological Studies:
- After the gross examination, several longitudinal cuts were made by a sharp knife.
- Tissue slices of 3 to 5 mm thick were preserved in 10% formal saline for 24hrs and then the tissues were passed through 30%, 50%, 70%, 90% and absolute alcohol, keeping for 12hrs in each of these.
- Clearing of the tissues were done with Xylene.
- Bathing of the tissues in the Paraffin bath LIL III.
- Embedding, Sectioning & Staining with H & E.

2.3.1 Lipid Staining:
- In this process we omit the steps of dehydration and clearing which will remove the lipids.
- We took Cryostat sections of fresh specimens and fixed it with 10% formal saline for staining with the fat stain i.e. Oil Red O.

2.3.2 Steps for Staining with Oil Red O:
- Fresh specimen from autopsy table was collected, dissected & cut into 1 mm size.
- It was smeared with the Cryostat solution & kept inside the cryostat at the temperature -20 to -30 °C.
- Frozen sections of 8 to 10 microns were cut & fixed in 10% formal saline.
- The stain must be freshly prepared from the stock solution and kept in a closed container.
- The formaline fixed slide is now rinsed with 60% isopropanol.
- Rinsed slide is stained with freshly prepared Oil Red O working solution for 15 min.
- Then again the slide is rinsed with 60% isopropanol.
- The slide is then dipped in Alum Haematoxylin for 5-7 dips.
- Then again rinsed with distilled water.
- Finally the slide is mounted in aqueous mountant i.e. glycerine jelly.

2.3.3 Results:
- Lipid → Red
- Nuclei → Blue

2.3.4 Reagent Formulae:
- Oil Red O Stock Stain:
  - Oil Red O → 0.5gm.
  - Isopropanol → 100 ml.

2.3.5 Oil Red O Working Solution:
- 30 ml of the stock solution is diluted with 20ml of distilled water, allowed to stand for 10 min & filtered into a coplin jar & covered immediately

III. Observation And Results:

PHOTOMICROGRAPHY NO. 4: CAPILLAR-HAEMORRHAGE, PARENCHYMAAL CORP IN ZONA FASCICULATA, SHRUNKEN CELLS IN ZONA GLOMERA, L.O.S.A WITH INCREASED NUCLEAR DENSITY.
200X HE STAIN
Table-I Histological Findings In Zona Glomerulosa

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SUICIDAL</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2) Cell size</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>3) Nuclear Prominence</td>
<td>Marked (Increased density)</td>
<td>Normal</td>
</tr>
<tr>
<td>3) Sinusoids</td>
<td>Prominent</td>
<td>Normal</td>
</tr>
<tr>
<td>4) Intracytoplasmic lipid depletion</td>
<td>Not marked</td>
<td>Not seen</td>
</tr>
<tr>
<td>5) Parenchymal cord like arrangement of cells</td>
<td>Not marked</td>
<td>Not seen</td>
</tr>
</tbody>
</table>

PHOTO MICROGRAPH NO : 2 SHOWING CAPSULAR HAEMORRHAGE IN SUICIDAL CASES , MARKED (H) .

CAPSULAR HAEMORRHAGE

PHOTOMICROGRAPH NO : 3 Showing Zonal Extension Of Zona Fasciculata
Histological Findings In Suicidal Cases & Control (H&E)
Table –II  Histological Findings In Zona Fasciculata :

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SUICIDAL</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Extension of ZF</td>
<td>Hyperplasia &amp; Hyper trophy.</td>
<td>Normal</td>
</tr>
<tr>
<td>2) Cell size</td>
<td>INCREASED</td>
<td>Normal</td>
</tr>
<tr>
<td>3) Nuclear Prominence</td>
<td>Nuclear density greater in inner zone</td>
<td>Normal</td>
</tr>
<tr>
<td>4) Sinusoids</td>
<td>Prominent</td>
<td>Normal</td>
</tr>
<tr>
<td>5) Intracytoplasmic lipid depletion</td>
<td>Varies from mild (+) patchy to moderate (+++) and finally extensive (+++)</td>
<td>Normal</td>
</tr>
<tr>
<td>6) Parenchymal cord like arrangement</td>
<td>INCREASED</td>
<td>No such arrangement seen</td>
</tr>
</tbody>
</table>

Photomicrograph No: 4 Showing Zonal Extension In Zona Fasciculata In Suicidal Cases
Zonal Extension

Photomicrograph No: 5 Showing degrees of lipid depletion in Zona Fasciculata (Suicidal cases)
Photomicrograph No: 6 Showing Sinusoidal Prominence In Suicidal Cases

Table III Showing Histological Findings In Zona Reticularis

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SUICIDAL</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Extension of zona reticularis</td>
<td>Slight increase</td>
<td>normal</td>
</tr>
<tr>
<td>2) Cell size</td>
<td>Increased</td>
<td>normal</td>
</tr>
<tr>
<td>3) Nuclear prominence</td>
<td>Not marked</td>
<td>normal</td>
</tr>
<tr>
<td>4) Sinusoids</td>
<td>Prominent</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Photomicrograph No: 7 Sinusoidal Prominence In Zona Reticularis
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Table – IV Histological Findings In Adrenal Medulla :

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SUICIDAL</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Extension of medulla</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>2) Cell size</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>3) Nuclear Prominence</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4) Sinusoids</td>
<td>Prominent</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Photomicrograph No – 8
Prominence Of Sinusoids In Adrenal Medulla

IV. Discussion :
Lipid accumulation in cells denote hypoactivity & lipid depletion indicates hyperactivity as seen in chronic stress. (Sayers et al., 1950).

In the present study Lipid depletion is seen in ZF in 90% cases which corroborates with the findings of Howe EE (2000), Kare M (1969), Willenberg H.S. et al & Rich A.R.et al (1998).

It is found that there is a hypertrophy in the inner region & hyperplasia in the outer region of ZF which is correlated with the findings of Yvonne M, Ulrich Lei et al (2006).

In 70% of the cases there is presence of prominent sinusoids which reflects the further vascular demand for the increased endocrine activities of adrenal in adaptation to new challenges to stress which corroborates with the findings of Howe EE (2000).

Increased parenchymal cord like formation was probably due to lipid depletion. BaccaroRB, Mendonce PO, Torres TE et al, 2007 found a growth factor FGF responsible for adrenocortical growth in response to stress.

According to Viard et al. (1994), it was observed that ACTH blocks progression of adrenocortical cells in G1 phase and inhibits DNA synthesis in S-phase, even in the presence of growth factors. After this inhibiting effect, ACTH induces expression of genes associated with cell cycle progression such as C-fos and C-jun. Bicknell et al (2001) stated that peptides from the N-terminal region of ACTH precursor causes hypertrophy and hyperplasia.
Spencer SJ et al (1992) stated that transforming growth factor β and activin play role in hypertrophy and hyperplasia. Peptide NPY is responsible for adrenal growth as stated by Bernet et al (1998). Spexime, a polypeptide has a strong inhibitory effect on the proliferative activity of adrenal cortex in stressful condition (Rucinski et al., 2010).

V. Conclusion

The present study concludes and supports the idea that chronic stress as in suicide usually induces adrenal growth (evidenced by zonal extension), lipid depletion & sinusoidal prominence which may have implications for forensic people in revealing the cause of unknown deaths.

References