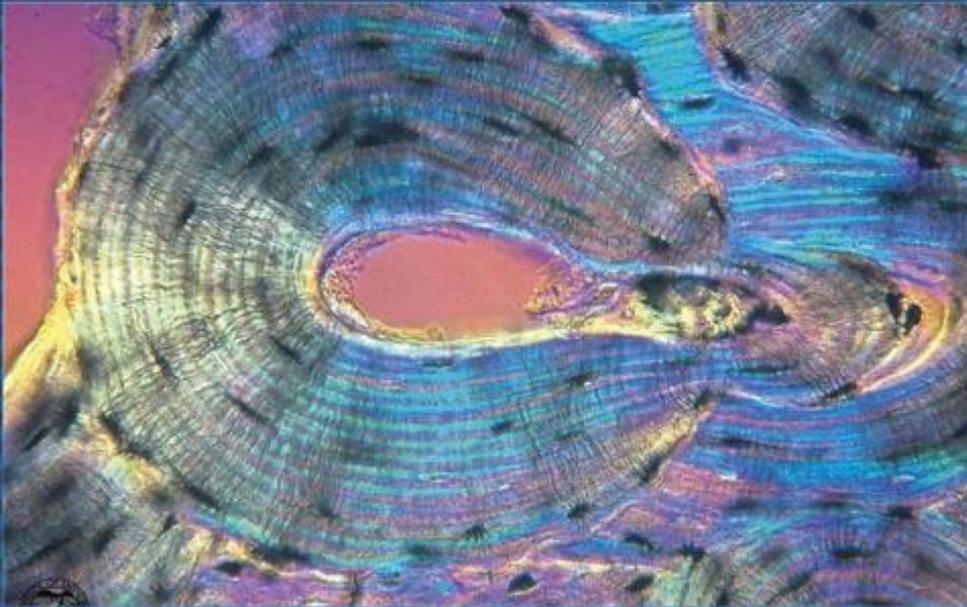




EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
HISTOLOGY & HISTOCHEMISTRY

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ISSN
2090-0775

WWW.EAJBS.EG.NET

Vol. 12 No. 2 (2020)



Progenitor Stem Cells Behavior in the Adrenal Gland of Acute-Stress Albino Rat Model: A Histological and Immunohistochemical Study

Nermeen Hosney Ahmed Lashine, Amany Shams, Shaima Mohamad Almasry, Huda El-Tahry and Marwa Abd El-kader*

Anatomy and Embryology Department, Faculty of Medicine, Mansoura University, Egypt

E.Mail : Drramym@gmail.com - dr_a.shams@yahoo.com - almasry.shaima@gmail.com - hudaeltahry@yahoo.com - marwa5566@mans.edu.eg

ARTICLE INFO

Article History

Received:12/10/2020

Accepted:22/12/2020

Keywords:

Stress, Stem Cell, Nestin, Chromogranin-A.

ABSTRACT

Background: The adrenal gland achieves a greater degree of plasticity in response to stress. This is attained through the proliferation and differentiation of the adult adrenal progenitors.

Aim of the work: This work aimed to assess the presence of adrenal progenitor cells and their behavior under acute stress and recovery.

Material and methods: Eighteen male rats (8 weeks, 200-250g) were equally divided into three groups: Control rats, Acute-Stress rats were exposed to restraint stress for 2 h without food and water during the stress period, recovered rats were exposed to the same pattern of stress, then housed in enriched environment supplied with running wheel for six days. After scarification, the blood samples were collected for plasma cortisol assessment, the glands were excised and processed for histological and immunohistochemical studies.

Result: The cortisol level was elevated in the acute-stress group, but returned to normal level in the recovered group. The adrenal glands of the acute-stress group showed vacuolations, congestion, and hemorrhage, however, the recovered group showed improvement in the gland histology. Both Nestin and GFAP expressions were detected mainly in the capsular and subcapsular regions and medulla of all groups. The expression chromogranin-A was markedly increased in both acute-stress and recovered groups as compared to the control group, and it was greatly higher in the acute-stress group as compared to the recovered group indicated increased chromaffin cell number.

Conclusion: In acute stress, Nestin progenitor cells of the adrenal gland were capable of differentiation into chromogranin-A expressing chromaffin cells.

INTRODUCTION

Stress is defined as a mechanism of adaptation that is essential for evolution and survival. The integrated endocrine, neural and immune responses to stress are necessary for homeostatic balance (Bornstein *et al.*, 2018). Under normal conditions, the adrenal medulla synthesizes and store their peptide hormones, and release only small amounts. However, during periods of acute stress larger quantities are secreted under the influence of the autonomic nervous system (Spencer and Deak, 2017).

Furthermore, the hypothalamus_pituitary_adrenal (HPA) axis plays an important role. The hypothalamus releases a corticotropin-releasing hormone to the pituitary gland that releases adrenocorticotrophic hormone (ACTH), which motivates the adrenal cortex, especially the zona fasciculata (ZF) to produce cortisol which is a stress hormone that rebalances the body functions and performances (Sunwoo *et al.*, 2019).

Maintenance of the body organs and tissues is achieved by the proliferation and differentiation of adult stem cells. These cells reside in several organs where they participate in the regeneration of their cells (Ge and Fuchs, 2018). Specific adult progenitor/stem cells are present in the cortical and medullary cells of the adrenal gland. They play an essential role in stress adaptation as they are responsible for the renewal of adrenal-specific cells (Ehrhart-Bornstein *et al.*, 2010; Walczak and Hammer, 2015).

The presence of stem cells in the adrenal medulla and cortex has been established in several studies. A pool of progenitor cells expressing Nestin protein was identified in the adrenal medulla. These progenitors actively participated in adaptation to stress by differentiation into chromaffin cells. Moreover, it was observed that Nestin-expressing cells were existed under the adrenal capsule and dispersed through the cortex (Steenblock *et al.*, 2017; Finco *et al.*, 2018). These cells are navigated centripetally until reaching the cortical-medullary margin (Chang *et al.*, 2013). The medullary progenitors have the ability to differentiate into three populations: glia, chromaffin, and neuronal cells, indicating their multipotent property. Similarly, the cortical Nestin-positive cells are capable of differentiation into steroidogenic cells (Rubin de Celis *et al.*, 2015).

The current experiment aimed to study the histological changes in the

adrenal gland in response to acute stress and recovery. Furthermore, to assess the presence of adrenal progenitor cells and their behavior under acute stress and recovery through evaluation of immunohistochemical expression of Nestin protein. Finally, to evaluate the ability of these progenitor cells to recruit new chromaffin and glial cells by evaluation of chromogranin-A and GFAP immunohistochemical expression.

MATERIALS AND METHODS

Animals:

Eighteen male rats aging 8 weeks and weighing 200-250g were obtained from the Medical Experimental Research Center of Mansoura University (MERC). The animals were kept six per cage under stable humidity ($60 \pm 5\%$), temperature (21 ± 2 °C), and light/dark cycles. They were allowed *ad libitum* access to food and water. The rats were maintained two weeks before the experiment for acclimatization and to ensure normal growth and behavior.

Induction of Stress Model:

The animals from the acute-stress group were individually placed in wire stainless mesh restrainers (5×7×12 cm in dimensions) - as described by Soliman (2006) - for 2 h/day (9.00 AM–11.00 AM) for one day without food and water during the stress period (Rubin de Celis *et al.*, 2015). This procedure effectively restricted the movement of the animals. The control group was housed in normal stainless cages (20 × 35 × 60 cm) (El-Desouki *et al.*, 2012).

Experimental Groups:

1- Group I (Control): six rats were maintained under stable conditions along the experimental period.

2- Group II (Acute-stress): six rats were exposed to the previous pattern of stress only one time and sacrificed on the 1st day.

3- Group III (Recovered group after acute stress): Six rats were exposed to

the same pattern of stress for only one day. Then, they were housed in an enriched environment supplied with a running wheel for six days and sacrificed on the 7th day.

The Sacrifice of Rats and Specimen's Collection:

After sacrifice, Blood samples were obtained by direct left ventricle puncture and stored at -20°C for plasma cortisone assessment. The adrenal glands were dissected, preserved in 10% buffered formalin, and processed for paraffin sections.

Assessment of Plasma Glucocorticoid:

The plasma level of glucocorticoid hormone was estimated by the radioimmunoassay (RIA) method (El-Farhan *et al.*, 2017).

Histological and Immunohistochemical Assessments:

The adrenal sections were cut at 5 µm and processed for Hematoxylin and eosin (Hx&E) staining (Bancroft and Gamble, 2008). For immunohistochemical staining, the adrenal sections were treated with 3% H₂O₂ and rinsed in phosphate-buffered saline (pH 7.4), then preserved with sodium citrate buffer (0.01 M, pH 6.0) in a water bath at 95°C for 30 min. after cooling to the room temperature, the slides were incubated with 1% BSA for 1h and then overnight at 4°C with the primary antibodies: anti-Nestin antibody (N5413, Sigma-Aldrich, 1:100 dilutions) (Bellafiore *et al.*, 2006; Klein *et al.*, 2014), Anti-GFAP antibody (ab7260, Abcam, 1:1000 dilutions) (Nedzvetskii *et al.*, 2016), Anti-chromogranin-A antibody (A0430, Abcam, 1:1500 dilutions) (Zhang *et al.*,

2018). The slides were incubated with HRP-conjugated secondary antibodies at 37°C for 30min, then treated with labeled streptavidin-biotin for another 30min. Finally, the diaminobenzidine was used to visualize the immunohistochemical reactions and the hematoxylin as a counterstain.

Morphometric Study and Statistical Analysis

Five fields from each adrenal gland were photographed using the Olympus® SC100 digital camera installed on Olympus® CX41light microscope. The optical density of the immunohistochemically stained sections (GFAP, chromogranin-A, and Nestin) were assessed using program NIH Image J program (National Institutes of Health, Bethesda, MD, USA) according to the program instructions.

Data were analyzed using the computer program SPSS v22 (Statistical package for social science version 22) for descriptive statistics in the form of mean±SD (standard deviation), and analytical statistics for comparison between the different groups using analysis of variance (ANOVA) test followed by post-hoc Tukey test for multiple comparisons. P value <0.05 was considered statistically significant.

RESULTS

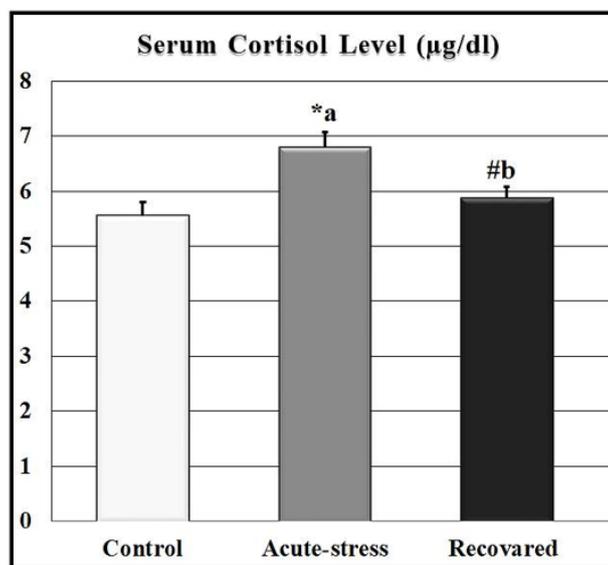
I- Plasma Cortisol Level:

Assessment of the plasma cortisol level revealed significant increases in the acute-stress group in comparison with the control group. This elevation was significantly decreased in the recovered group (Table 1, Histogram 1).

Table 1: Effects of acute stress and recovery on the plasma cortisol level

Variables	Control	Acute-Stress	Recovered Group	P value
Cortisol level (µg/dl)	5.554±0.252	6.76±0.289	5.87±0.211	P1=0.002 P2=0.014 P3=0.366

The above values are expressed as mean ±SD using ANOVA test. P1:P3 comprise post-hoc Tukey test where **P1**: acute stress versus control, **P2**: recovered group versus acute stress, **P3**: recovered group versus control.



Histogram 1: Histogram illustrating the mean plasma cortisol level. It is significantly increased in the acute-stress group when compared to the control group and decreased in the recovered group compared to the acute-stress group. #p<0.05, *p<0.01. **a**: versus control. **b**: versus acute stress.

II- Hx&E-Stained Sections:

Control group

The adrenal gland represented a central pale medulla and the outer dark cortex. The cortex consisted of: zona glomerulosa (ZG, its cells form small nests) (Fig. 1A), zona fasciculata (ZF, its cells form parallel cords disjoined by blood sinusoids) (Fig. 1B), and zona reticularis (ZR, its cells form anastomosing cords) from outside to inside. The medulla was formed of follicles of chromaffin cells with vesicular nuclei (Fig. 1C).

Acute stress group

The cells of ZG were swollen due to edema. Vacuolations and pyknotic nuclei were detected (Fig. 1D). The ZF and ZR showed dilatation of the blood sinusoids and blood engorgement (Fig. 1E). In the medulla, chromaffin cells were crowded, swollen and showed many vacuoles. Narrowing of the follicular lumen and the

interfollicular spaces, vascular congestion, and hemorrhage was detected (Fig. 1F).

Recovered group after acute stress

Comparing to the acute stress group, sections of the adrenal gland of this group showed preserved adrenal architecture in some rats and loss of central medullary architecture in others. The ZG and ZF cells were showed many vacuolations, hemorrhage, and degenerated nuclei (Fig. 1G, 1H). The ZR showed dilatation and an increased number of blood sinusoids. There were vacuolations in the medulla and dilatation of the blood sinusoids (Fig. 1I).

II- Immunohistochemical Stained Sections:

A) Immunohistochemistry for Nestin

The Nestin immunostaining appeared as a positive cytoplasmic reaction mainly in the capsular and subcapsular regions and medulla of the

control (Fig. 2A, 2B), acute-stress (Fig. 2C, 2D), and recovered (Fig. 2E, 2F) groups. There were non-significant differences in the optical density of the total Nestin positive cells between all studied groups (Table 2, Histogram 2A).

B) Immunohistochemistry for Chromogranin-A

The positive immunoreaction of chromogranin-A in the control group appeared as dispersed cytoplasmic reactions in the medullary cells (Fig. 3A). The acute-stress group showed a strong positive immunoreaction in the medullary cells (Fig. 3B) with a significant increase in the optical density of chromogranin-A as compared to the control group. The recovered group represented a moderate positive

immunoreaction (Fig. 3C) with a marked decrease in the optical density as compared to the acute-stress group and a significant increase as compared to the control group (Table 2, Histogram 2B).

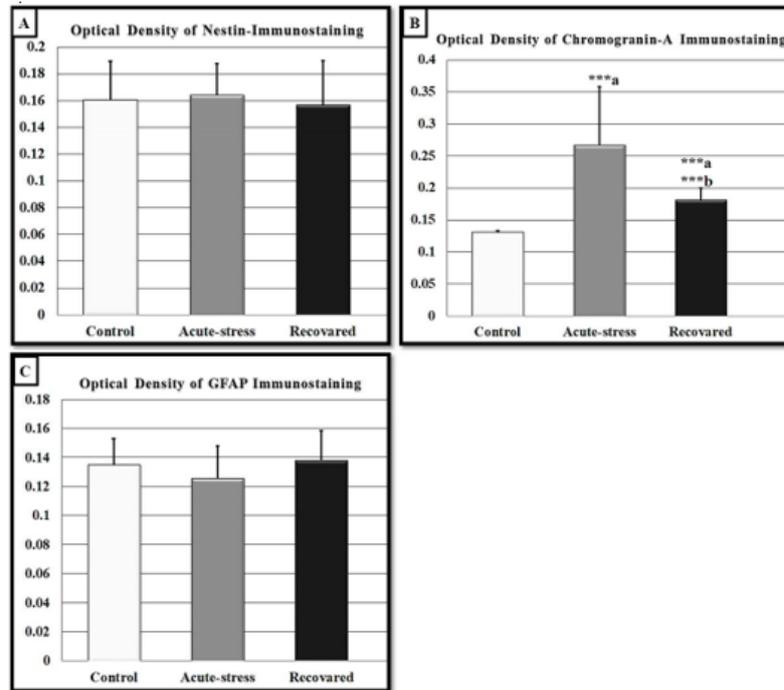
C) Immunohistochemistry for GFAP

The GFAP immunostaining appeared as a strong positive cytoplasmic reaction in the capsular and subcapsular regions and medulla of the control (Fig. 4A, 4B), acute-stress (Fig. 4C, 4D), and recovered (Fig. 4E, 4F) groups. Cells of ZG, ZF, and ZR weakly express the immune stain. There were non-significant differences in the optical density of the total GFAP positive cells between the studied groups (Table 2, Histogram 2C).

Table 2: The Mean Optical Density of the Nestin, Chromogranin-A, and GFAP Adrenal Gland Immunostain.

Variables	Control	Acute-Stress	Recovered	<i>P value</i>
Optical Density of Nestin Immunostain	0.160±0.029	0.163±0.018	0.156±0.016	<i>P1=0.840</i> <i>P2=0.749</i> <i>P3=0.603</i>
Optical Density of Chromogranin-A Immunostain	0.130±0.002	0.266±0.092	0.180±0.019	<i>P1=0.000</i> <i>P2=0.000</i> <i>P3=0.000</i>
Optical Density of GFAP Immunostain	0.134±0.018	0.125±0.022	0.137±0.020	<i>P1=0.448</i> <i>P2=0.315</i> <i>P3=0.799</i>

The above values are expressed as mean ±SD using ANOVA test. P1:P3 comprise post-hoc Tukey test where **P1**: acute stress versus control, **P2**: recovered group versus acute stress, **P3**: recovered group versus control



Histogram 2: A) Histogram illustrating non-significant differences in the mean Nestin optical density of the studied groups. B) Histogram illustrating a significant increase in the Chromogranin-A mean optical density of both acute-stress and recovered groups compared to the control group and decrease in the recovered group compared to the acute-stress group. C) Histogram illustrating non-significant differences in the mean GFAP optical density of the studied groups. *** $p < 0.0001$. a: versus control. b: versus acute stress.

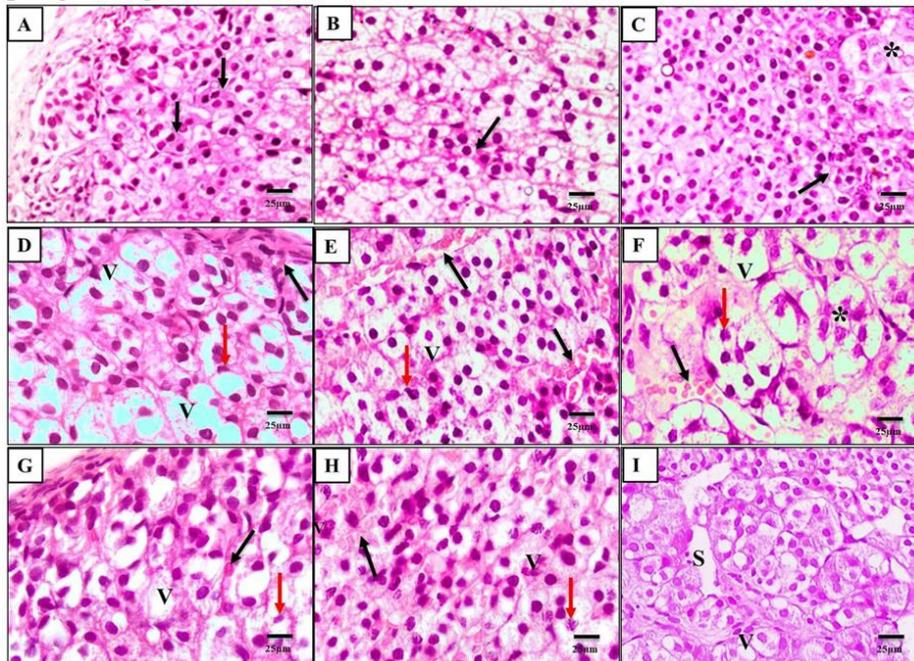


Fig. 1: Showing the Hx&E-stained adrenal gland sections. The photomicrographs of the control rat demonstrating ZG cells arranged in small nests (A), ZF cells are arranged in parallel cords (B), ZR cells are arranged in anastomosing cords, and the medulla formed of follicles of chromaffin cells (*) with vesicular nuclei (C). The photomicrographs of the acute-stress rat demonstrating vacuolations (v), hemorrhage (black arrows), and degenerated nuclei (red arrows) in the ZG (D), ZF (E), and medulla (F). The medullary chromaffin cells (*) are swollen with narrowing of the follicular lumen and the interfollicular spaces. The photomicrographs of the recovered rat showing improvement in the adrenal architecture with few vacuolations (v), hemorrhage (black arrows), and degenerated nuclei (red arrows) in the ZG (G), ZF (H), dilated sinusoid (S), and vacuolations (v) in the medullary chromaffin cells (*) (I).

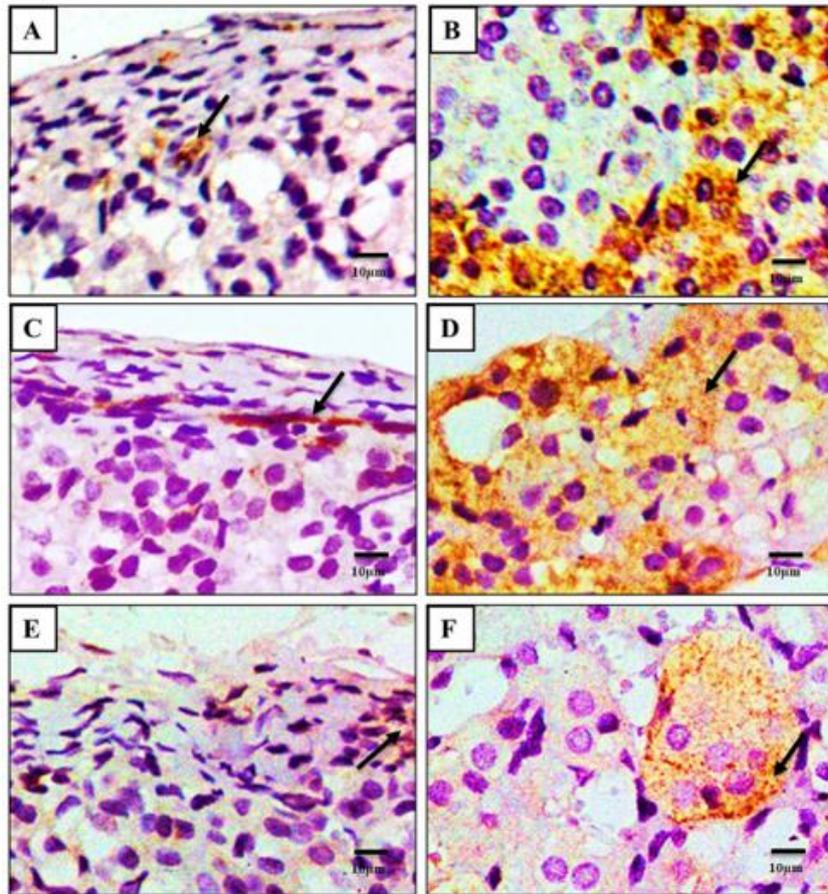


Fig. 2: Showing the Nestin immunostained adrenal gland sections. The photomicrographs of the control rat demonstrating dotted reactions in the capsular and subcapsular regions (A) and a strong reaction in the medulla (B). The photomicrographs of the acute-stress rat demonstrating strong positive reactions in the capsular and subcapsular regions (C) and medulla (D). The photomicrographs of the recovered rat showing positive reactions in the capsular and subcapsular regions (E) and medulla (F).

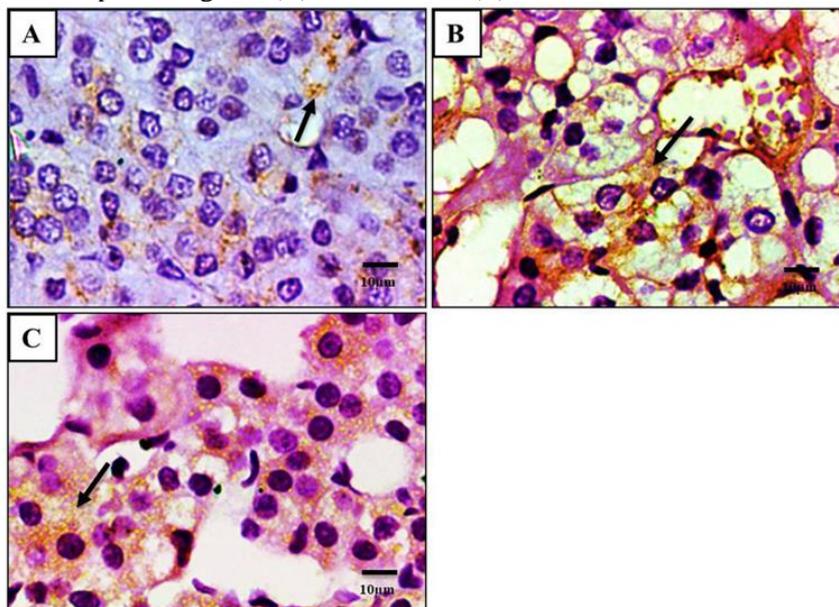


Fig. 3: Showing the Chromogranin-A immunostained adrenal gland sections. The photomicrographs of the control rat demonstrating diffuse and dotted cytoplasmic reaction in the medullary cells (A). The photomicrographs of the acute-stress rat demonstrating a strong positive immunoreaction in the cytoplasm of the medullary cells (B). The photomicrographs of the recovered rat showing a moderate positive immunoreaction (C).

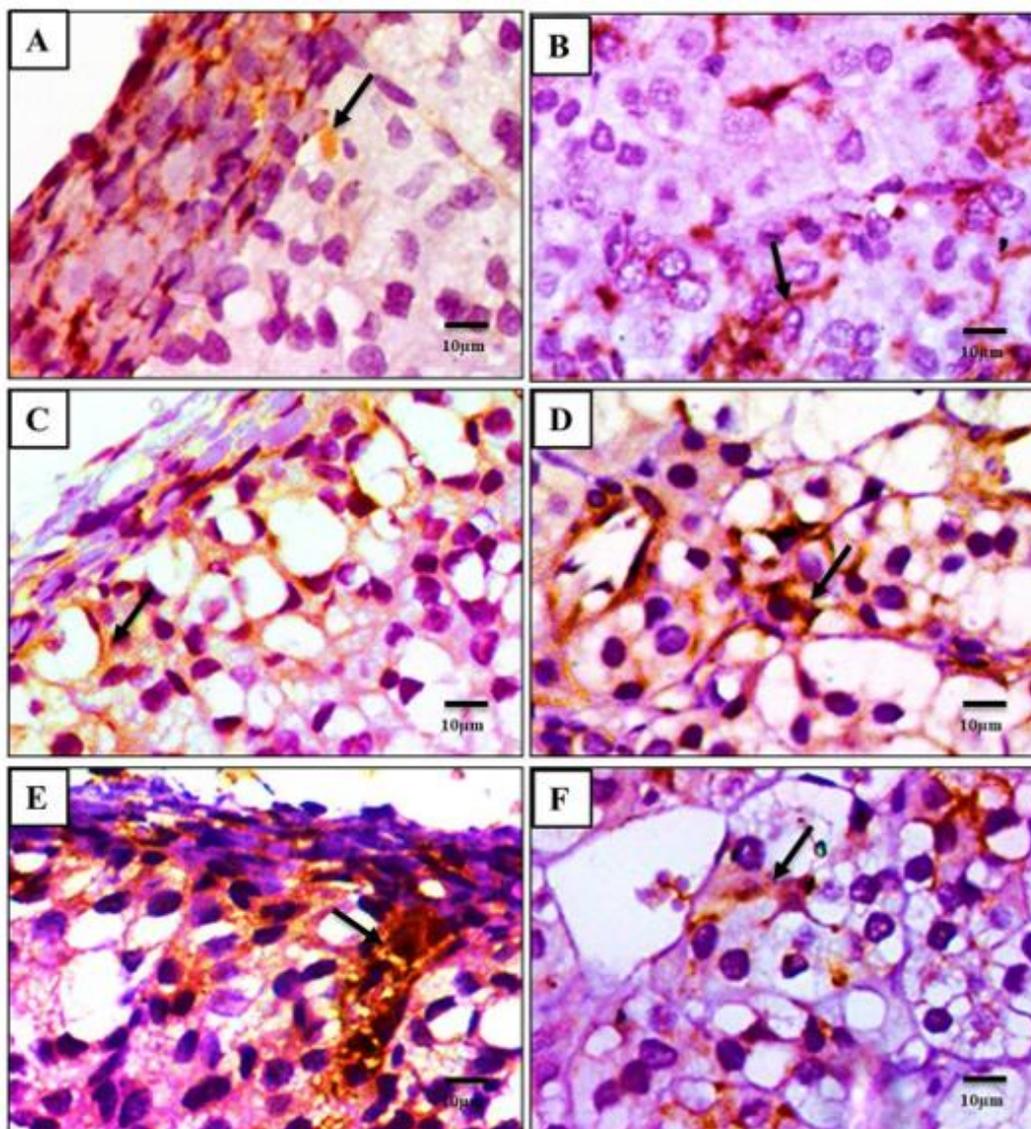


Fig. 4: Showing the GFAP immunostained adrenal gland sections. The photomicrographs of the control rat demonstrating a strong positive reaction in the capsular region (A) and medulla (B). The photomicrographs of the acute-stress rat demonstrating moderate positive reactions in the capsular and subcapsular regions (C) and medulla (D). The photomicrographs of the recovered rat showing positive reactions in the capsular and subcapsular regions (E) and medulla (F).

DISCUSSION

The current study is interesting for two reasons; first, it extends our knowledge on the effect of acute stress on the adrenal gland, second, it assesses the presence of progenitor/stem cells in the adrenal gland and their ability to switch into new chromaffin cells.

The induction of acute stress was performed by restraining the rats individually in wire stainless mesh restrainers for 2h. Restraint stress is a common stressor in rodents that well documented to induce cognitive,

neuroendocrine, and immune function impairments (Mo *et al.*, 2019).

Endocrine and neural responses to stress involve activation of both the HPA and the sympathoadrenal system with subsequent release of the adrenocortical glucocorticoids as well as medullary catecholamines (McCarty, 2016). The current study demonstrated a significant increase in plasma cortisol levels of the acute-stress group that returned to the control level in the recovered group. Elevation of the corticosterone level in response to stress

was previously documented in different acute-stress models (Rostamkhani *et al.*, 2012; Jameel *et al.*, 2014; Hwang *et al.*, 2019; Tsukada *et al.*, 2021).

In the current study, the acute-stress group showed many pathological features in the adrenal cortices in the form of vacuolations, pyknosis, vascular congestion, and hemorrhage. The recovered group showed preserved adrenal architecture in some rats and some degenerative changes in others. Howard (2018) reported that vacuolar degeneration of the ZG and ZR is considered to be a nonspecific lesion associated with stress, disease states, or trauma. Adrenal congestion and hemorrhage could be explained by an increased level of ACTH which stimulates the release of catecholamines that increases the blood flow to the adrenal. Also, an increase in ACTH stimulates the formation of prostaglandins leading to congestion (Zidan and Elnegris, 2013). Hemorrhage is more likely to occur due to endothelial cell damage caused by endotoxins released with the increased gland activity or hypoxia due to gland congestion (Karki *et al.*, 2021).

In the current study, the positive expression of Nestin was detected as diffuse immunoreaction in the capsular and subcapsular regions (ZG layer), scattered in the ZF and ZR, and strong reaction in the medulla. In consistence with our results, Steenblock *et al.* (2018) declared that the Nestin positive cells were mainly located in the medulla and ZG, while scanty Nestin expressing cells were dispersed in the ZF and ZG. Chung *et al.* (2009) and Langton *et al.* (2018) have documented that Nestin positive cells exist in the adult human adrenal medulla.

Regarding the optical density of total adrenal Nestin immunoreaction, there were non-significant differences between the studied groups. This could be explained by the findings of Rubin de Celli *et al.* (2015) who reported that, at resting conditions, Nestin expressing cells proliferate at a low rate, but they

respond with enhanced proliferation to the extremely challenging circumstances, even if one day of stress. However, after differentiation, Nestin positive expression was reduced. In line with our results, Steenblock *et al.* (2018) observed that under immobilization stress the total number of Nestin positive cells in the cortex was unchanged, while the number of capsular Nestin positive cells was reduced, signifying rapid centripetal migration in response to stress.

The chromogranin-A is the major protein for the adrenomedullary chromaffin cells (Machado *et al.*, 2010; Abbineni *et al.*, 2019). In the present study, the positive immune reaction for chromogranin-A was detected in the cytoplasm of the medullary cells. The expression was significantly increased in both acute-stress and recovered groups as compared to the control group. However, it was significantly lower in the recovered groups as compared to the acute-stress group. Under normal conditions, Nestin-positive progenitors are able to differentiate into glial, neuronal, and chromaffin cells. However, under stress, in which the adrenal shows an extreme adaptive reaction, they favorably differentiate into chromaffin cells. Furthermore, it was documented that the chromaffin progenitor cells have the ability to self-renew in culture and differentiate into hormone-producing chromaffin cells (Vukicevic *et al.*, 2012; Saxena *et al.*, 2013; Rubin de Celli's *et al.*, 2015).

There is no cellular co-localization of chromogranin-A and Nestin positive detected in any adult adrenal studies, indicating that Nestin is not expressed by differentiated chromaffin cells. So, the increased chromogranin-A expression in spite of the non-significant increase in Nestin could be explained by proliferation and differentiation of the adrenal progenitor cells in response to acute stress (Rubin de Celli *et al.*, 2015).

Unlike chromogranin-A, approximately 62% of the medullary Nestin expressing cells were co-expressing GFAP, indicating similar properties of these progenitor cells with Nestin stem cells. In the current study, the immunoreaction of GFAP is strong in the capsule and medulla, and moderate in the ZG, ZF, and ZR. There were non-significant differences in the GFAP immunoreaction between the studied groups. These results could be explained by the progenitor Nestin stem cells differentiate into glial cells under normal conditions. On the contrary, under stressful circumstances, the differentiation progressed considerably toward the chromaffin lineage producing chromogranin-A expressing chromaffin cells (Rubin de Celli *et al.*, 2015).

CONCLUSION

It could be concluded that, in acute stress, Nestin progenitor cells of the adrenal gland increased and were able to differentiate into chromaffin cells.

Ethical Approval: This study was approved by the institutional research board (IRB) of the Faculty of Medicine, Mansoura University, Egypt (code number: MD.18.03.17).

REFERENCES

- Abbineni, P. S.; Bittner, M. A.; Axelrod, D. and Holz, R. W. (2019) Chromogranin A, the major lumenal protein in chromaffin granules, controls fusion pore expansion. *The Journal of general physiology*, 151(2) pp: 118–130.
- Bancroft, J. D. and Gamble, M. (2008) Theory and practice of histological techniques. sixth ed. Churchill Livingstone Elsevier, Philadelphia.
- Bellafiore, M.; Sivverini, G.; Cappello, F.; David, S.; Palma, A.; Farina, F. and et al. (2006) Research of cardiomyocyte precursors in adult rat heart. *Tissue and Cell*, 38(6) pp: 345-351.
- Bornstein, S. R.; Steenblock, C.; Chrousos, G. P.; Schally, A. V.; Beuschlein, F.; Kline, G. and et al., (2018) Stress-inducible-stem cells: a new view on endocrine, metabolic and mental disease?. *Molecular psychiatry*, 24(1) pp: 2-9.
- Chang, S. P.; Morrison, H. D.; Nilsson, F.; Kenyon, C. J.; West, J. D. and Morley, S. D. (2013) Cell proliferation, movement and differentiation during maintenance of the adult mouse adrenal cortex. *PloS one*, 8(12), e81865.
- Chung, K. F.; Sicard, F.; Vukicevic, V.; Hermann, A.; Storch, A.; Huttner, W. B. and et al. (2009) Isolation of neural crest derived chromaffin progenitors from adult adrenal medulla. *Stem cells*, 27(10) pp: 2602-2613.
- Ehrhart-Bornstein, M.; Vukicevic, V.; Chung, K. F.; Ahmad, M. and Bornstein, S. R. (2010) Chromaffin progenitor cells from the adrenal medulla. *Cellular and molecular neurobiology*, 30(8) pp: 1417-1423.
- El-Desouki, N. I.; El-Refaiy, A. I.; Afifi, D. F. and Abdel-Kader, A. A. (2012) Histological, histochemical, and immunohistochemical studies of the cardiac muscle of the albino rat under immobilization stress and the curative role of diazepam. *The Egyptian Journal of Experimental Biology (Zoology)*, 8(2) pp: 273-285.
- El-Farhan, N.; Rees, D. A. and Evans, C. (2017) Measuring cortisol in serum, urine and saliva—are our assays good enough? *Annals of clinical biochemistry*, 54(3) pp: 308-322.
- Finco, I.; Lerario, A. M. and Hammer, G. D. (2018) Sonic hedgehog and WNT signaling promote adrenal gland regeneration in

- male mice. *Endocrinology*, 159(2) pp: 579-596.
- Ge, Y. and Fuchs, E. (2018) stretching the limits: from homeostasis to stem cell plasticity in wound healing and cancer. *Nature reviews. Genetics*, 19(5) pp: 311-325.
- Howard, E. B. (2018) Miscellaneous diseases. In *Pathobiology of marine mammal diseases* (pp: 163-225). CRC Press.
- Hwang, K. A.; Hwang, Y. J.; Hwang, I. G.; Song, J. and Jun Kim Y. (2019) Low temperature-aged garlic extract suppresses psychological stress by modulation of stress hormones and oxidative stress response in brain. *Journal of the Chinese Medical Association*, 82(3) pp: 191-195.
- Jameel, M. K.; Joshi, A. R.; Dawane, J.; Padwal, M.; Joshi, A. R.; Pandit, V. A. and et al. (2014) Effect of various physical stress models on serum cortisol level in wistar rats. *Journal of clinical and diagnostic research*, 8(3) pp: 181-183.
- Karki, B. R.; Sedhai, Y. R. and Bokhari, S. R. A. (2021) Waterhouse-Friderichsen Syndrome. In: *StatPearls [Internet]*. Treasure Island (FL): Stat Pearls Publishing.
- Klein, D.; Meissner, N.; Kleff, V.; Jastrow, H.; Yamaguchi, M.; Ergün, S., and et al. (2014) Nestin (+) tissue-resident multipotent stem cells contribute to tumor progression by differentiating into pericytes and smooth muscle cells resulting in blood vessel remodeling. *Frontiers in oncology*, 4: 169.
- Langton, K.; Gruber, M.; Masjkur, J.; Steenblock, C.; Peitzsch, M.; Meinel, J. and et al. (2018) Hypertensive crisis in pregnancy due to a metamorphosing pheochromocytoma with post-delivery Cushing's syndrome. *Gynecological Endocrinology*, 34(1) pp: 20-24.
- Machado, J. D.; Díaz-Vera, J.; Domínguez, N.; Álvarez, C. M.; Pardo, M. R. and Borges, R. (2010) Chromogranins A and B as regulators of vesicle cargo and exocytosis. *Cellular and molecular neurobiology*, 30(8) pp: 1181-1187.
- McCarty, R. (2016) Learning about stress: neural, endocrine and behavioral adaptations. *Stress*, 19(5) pp: 449-475.
- Mo, C.; Renoir, T. and Hannan, A. J. (2019) Stress and Glucocorticoids as Experience-Dependent Modulators of Huntington's disease. In *Stress: Physiology, Biochemistry, and Pathology* (pp: 243-278). Academic Press.
- Nedzvetskii, V. S.; Pryshchepa, I. V.; Tykhomyrov, A. A. and Baydas, G. (2016) Inhibition of Reactive Gliosis in the Retina of Rats with Streptozotocin-Induced Diabetes under the Action of Hydrated C60 Fullerene. *Neurophysiology*, 48(2) pp: 130-140.
- Rostamkhani, F.; Zardooz, H.; Zahediasl, S. and Farrokhi, B. (2012) Comparison of the effects of acute and chronic psychological stress on metabolic features in rats. *Journal of Zhejiang university science B*, 13(11) pp: 904-912.
- Rubin de Celis, M. F.; Garcia-Martin, R.; Wittig, D.; Valencia, G. D.; Enikolopov, G.; Funk, R. H. and et al. (2015) Multipotent Glia-Like Stem Cells Mediate Stress Adaptation. *Stem Cells*, 33(6) pp: 2037-2051.
- Saxena, S.; Wahl, J.; Huber-Lang, M. S.; Stadel, D.; Braubach, P.; Debatin, K. M. and et al. (2013) Generation of murine sympathoadrenergic progenitor-like cells from embryonic stem cells and postnatal adrenal

- glands. *PLoS One*, 8(5): e64454.
- Soliman, A. A. (2006) Effect of stress on the mammary gland of lactating albino rat: a light and electron microscopic study. *Egyptian Journal of Histology*, 29(2) pp: 259-268.
- Spencer, R. L. and Deak, T. (2017) A users guide to HPA axis research. *Physiology & behavior*, 178, pp: 43–65.
- Steenblock, C.; de Celis, M. F. R.; Androutsellis-Theotokis, A.; Sue, M.; Silva, L. F. D.; Eisenhofer, G. and et al. (2017) Adrenal cortical and chromaffin stem cells: Is there a common progeny related to stress adaptation? *Molecular and cellular endocrinology*, 441, pp: 156-163.
- Steenblock, C.; de Celis, M. F. R.; Silva, L. F. D.; Pawolski, V.; Brennand, A.; Werdermann, M. and et al. (2018) Isolation and characterization of adrenocortical progenitors involved in the adaptation to stress. *Proceedings of the National Academy of Sciences*, 115(51) pp: 12997-13002.
- Sunwoo, S. H.; Lee, J. S.; Bae, S.; Shin, Y. J.; Kim, C. S.; Joo, S. Y. and et al. (2019) Chronic and acute stress monitoring by electrophysiological signals from adrenal gland. *Proceedings of the National Academy of Sciences of the United States of America*, 116(4) pp: 1146–1151.
- Tsukada, M.; Ikemoto, H.; Lee, X. P.; Takaki, T.; Tsuchiya, N.; Mizuno, K. and et al (2021) Kamikihito, a traditional Japanese Kampo medicine, increases the secretion of oxytocin in rats with acute stress. *Journal of Ethnopharmacology*, 276: 114218.
- Vukicevic, V.; Schmid, J.; Hermann, A.; Lange, S.; Qin, N.; Gebauer, L. and et al. (2012) Differentiation of chromaffin progenitor cells to dopaminergic neurons. *Cell transplantation*, 21(11) pp: 2471-2486.
- Walczak, E. M. and Hammer, G. D. (2015) Regulation of the adrenocortical stem cell niche: implications for disease. *Nature Reviews Endocrinology*, 11(1) pp: 14-28.
- Zhang, D.; Xie, C.; Wang, R.; Yang, Q.; Chen, H.; Ling, S. and et al. (2018) Effective preparation of a monoclonal antibody against human chromogranin A for immunohistochemical diagnosis. *BMC biotechnology*, 18(1) pp: 25.
- Zidan, R. A. and Elnegriss, H. M. (2013) A histological study on the effect of noise on the adrenal cortex of adult male guinea pigs and the possible role of combined vitamins (A, C, and E) supplementation. *Egyptian Journal of Histology*, 36(4) pp: 857-868.

ARABIC SUMMARY

سلوك خلايا السلف الجذعية في الغدة الكظرية لنموذج الضغط العصبي الحاد في الجرذ الأبيض: دراسة نسيجية وكيميائية مناعية

نرمين حسني أحمد لاشين وأمني محمد شمس الدين وشيماء محمد المصري وهدى محمد الطاهري
ومروة السيد عبد القادر
قسم التشريخ والاجنة-- كلية الطب- جامعة المنصورة

المقدمة: تحقق الغدة الكظرية درجة كبيرة من الليونة في الاستجابة للضغط العصبي. يتم تحقيق ذلك من خلال تكاثر وتمايز خلايا أسلاف الغدة الكظرية.

هدف الدراسة: يهدف هذا العمل إلى تقييم وجود خلايا السلف الجذعية في الغدة الكظرية وسلوكها في ظل الضغط العصبي الحاد والتعافي.

المواد والطرق المستخدمة: تم تقسيم ثمانية عشر ذكرا من الجرذان البالغة بالتساوي إلى ثلاث مجموعات: المجموعة الأولى (المجموعة الضابطة)، المجموعة الثانية (المجموعة المعرضة للضغط العصبي الحاد) تم تعريضها للضغط العصبي لمدة ساعتين يوميا لمدة يوم واحد ثم ذبحت. المجموعة الثالثة (مجموعة التعافي من الضغط العصبي) تعرضت هذه المجموعة لنفس النمط من الضغط العصبي الحاد ثم انتقلت الي بيئة خالية من الضغط العصبي ثم ذبحت في اليوم السابع من بداية التجربة. تم جمع عينات الدم لتقييم مستوى الكورتيزول في البلازما، وتم استئصال الغدد الكظرية ومعالجتها من أجل الدراسات النسيجية والكيميائية المناعية.

النتائج: ارتفع مستوى الكورتيزول في مجموعة الضغط العصبي الحاد، لكنه عاد إلى المستوى الطبيعي في المجموعة التي تعافت. أظهرت الغدد الكظرية لمجموعة الضغط العصبي الحاد فجوات واحتقان ونزيف، ومع ذلك، أظهرت المجموعة المتعافية تحسناً في أنسجة الغدة. نتيجة التعرض للضغط العصبي تحولت خلايا النستن الي الخلايا المنتجة للادرنالين و النورادرنالين لمواجهة ازدياد حاجة الجسم الي هرمونات الغده الكظرية.

الاستنتاج: نستخلص من هذه الدراسة ان هناك صلة قوية للغاية بين خلايا النستن وتعرض الجسم للضغط العصبي.