ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.702.710

Research Article
Histochemical, Immunohistochemical and Ultrastructural Identification and Characterization of Neurosecretory Cells of Pineal Gland

[1]Shehata Mohamed Mohamed Soliman, [2]Khaled Mohamed Mazher, [2]Taghreed Mohamed Nabil, [2]Usama Kamal Moawad and [2]Randa Mohamed Hassan

[1]Department of Cytology and Histology, Faculty of Veterinary Medicine, El-Minia University, El-Minia, Egypt
[2]Department of Cytology and Histology, Faculty of Veterinary Medicine, Beni-Suef University, 62511 Beni-Suef, Egypt

Abstract

Background and Objective: The neuroendocrine system is a variety of nervous and endocrine tissues distributed throughout the body either organized or associated with other organs. The present study aimed to identify and characterize the features of pinealocytes as one of these neuroendocrine cells (NECs) in order to establish a histological scheme. **Materials and Methods:** A total of 40 adult apparently healthy New Zealand rabbits of both sexes were used. Pineal glands were obtained and subjected to the following reactions; general, specific, highly specific and immunohistochemical reactions for neuroendocrine cells in addition to the ultrastructural examinations. **Results:** The two types of pinealocytes; Pinealocytes type I and II were contained electron dense secretory granules. These granules were reacted positively with Grimelius silver impregnation, Lead Hematoxylin and OFG stains. The pinealocytes showed moderate reactions with Gomori's aldehyde fuchsin and aldehyde thionine stains. By using of Gomori's chrome alum hematoxylin and performic acid alcian blue stains, the cytoplasmic granules showed intense reactions. Immunohistochemically, the pinealocytes were subjected to anti-CgA antibody, anti-NSE antibody and anti-melatonin antibody gave positive reactions. **Conclusion:** It can be concluded that a model design for histological scheme that could be beneficial for biologists especially histologists in identification and characterization of neuroendocrine cells distributed throughout the body.

Key words: Pineal gland, neurosecretory cells, pinealocytes, histochemical, immunohistochemical

Citation: Shehata Mohamed Mohamed Soliman, Khaled Mohamed Mazher, Taghreed Mohamed Nabil, Usama Kamal Moawad and Randa Mohamed Hassan, 2019. Histochemical, immunohistochemical and ultrastructural identification and characterization of neurosecretory cells of pineal gland. Asian J. Biol. Sci., 12: 702-710.

Corresponding Author: Usama Kamal Moawad, Department of Cytology and Histology, Faculty of Veterinary Medicine, Beni-Suef University, 62511 Beni-Suef, Egypt Tel/Fax: 00 20 822 327982

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The neuroendocrine system represented by a group of various nervous and endocrine tissues forming a complex regulatory mechanism depending upon chemical signaling between them[1]. It composed a variety of cells distributed throughout the body either organized (in hypothalamus, thalamus, ganglia and pineal gland) or associated with other organs (as gastrointestinal and respiratory tract). These cells were characterized by the presence of numerous large dense core vesicles produced predominantly in the soma then travel to their releasing sites[2]. Additionally, these cells were able to synthesize many amines and peptides[3]. Neuro-secretory cells (NSCs) play an essential role in orchestrating many vital body functions; reproduction, metabolism, growth and regulation of density of bones as well as skin color[2].

The pineal gland was a part of the neuro-endocrine-reproductive axis[4]. Functions of pinealocytes were influenced by many functional modifications of components of this axis. These cells expressed some neuronal markers; neurofilaments, synaptophysin, tubulin and neural adhesion molecule[5].

Melatonin, the pineal hormone, coordinates the reproduction with the seasons of the year. The photoneuroendocrine system represented mainly by the pineal gland, where the melatonin hormone conveys a chemical signals to the hypothalamo-pituitary-gonadal axis about the day length[6].

The current investigation aimed to establish a methodology for identification and characterization of the neurosecretory cells of pineal gland as one of neuroendocrine system using general, specific, highly specific, immunohistochemical reactions of NECs in addition to, the ultrastructural examinations. This paper may be a preliminary trial in designing a histological schemes help histologists in identification and characterization of all neurosecretory cells throughout the body.

MATERIALS AND METHODS

The present work was conducted at the Department of Histology and Cytology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. This study lasted for a year from September 2017-August, 2018. The use of animals in the current study was approved by the Experimental Animal Ethics Committee, Faculty of Science, Beni-Suef University, Egypt (The approval number is BSU/FS/2016/14).

For light microscopic examination: A total of 40 adult apparently healthy New Zealand rabbits of both sexes were used.

Sampling: Immediately after slaughtering the rabbits, the dorsal portions of the skulls were removed. Heads including brains were immersed in the used fixatives for several hours to allow hardening of the brains. The brain samples were cut sagittally into 2 similar halves for obtaining the pineal gland.

Fixation: The pineal gland samples were examined grossly for any pathological lesions. Only were apparently normal ones taken and immediately transferred to the following fixatives; 20% formalin and Bouin's fluid.

Processing and sectioning: Samples were dehydrated using ascending grades of alcohol, cleared in xylene then impregnated in soft paraplast and lastly embedded in hard paraplast. Sections of about 4-6 μm were obtained, mounted onto glass slides, immersed in xylene to remove the paraplast, rehydrated in descending grades of alcohols then stained.

Staining:

General Stains: 1-Harris Hematoxylin and Eosin. 2-Toluidine blue.

Nonspecific reactions:

- Grimelius silver impregnation technique
- Lead hematoxylin stain
- OFG method

Specific reactions:

- Gomori's aldehyde fuchsin stain (AF)
- Aldehyde thionine stain (AT)
- Gomori's chrome alum hematoxylin phloxine stain (CAH)
- Performic acid alcian blue stain (PA-AB)

Immunohistochemical localization

Chromogranin A (CgA) using anti-CgA clone DAK-A3: By application of PAP technique using monoclonal mouse anti-human antibody that presented from DAKO, Glostrup, Denmark.

Neuron specific enolase using anti-NSE clone BBS/NC/VI-H14: By application of PAP technique using monoclonal mouse anti-human antibody on the brain that presented from DAKO, Glostrup, Denmark[7].

Highly specific reactions

Immunohistochemistry: Application of PAP technique using polyclonal mouse anti-human "Anti-Melatonin" (Mt,

N-Acetyle-5-Methoxytryptamine) antibody for detection of specific melatonin hormone granules, that presented from US Biological, United States.

The above mentioned fixatives and stains were applied as outlined by Bancroft and Stevens[8] and Bancroft and Gamble[9].

For transmission electron microscopic examination: Four samples were obtained from Pineal glands of adult apparently healthy New Zealand rabbits of both sexes. The obtained samples were cut into 1-3 mm thick specimens, prefixed in 3% glutaraldehyde in phosphate buffer solution, washed in the same phosphate buffer several times and kept overnight[10] at 4°C. Specimens were post-fixed in 1% osmium tetroxide, rinsed three times in distilled water, dehydrated, cleared and then embedded in Epon. Semi-thin sections were stained using Toluidine Blue stain and examined by the light microscopy[11]. Ultra-thin sections were contrasted using 5% uranyl acetate followed by lead citrate stain[12], then examined by using JEOL (JEM-1400 TEM) at the candidate magnification in the Electron Microscopy Unit, Faculty of Agriculture, Cairo University, Egypt.

RESULTS

Characterization

General structure

Light microscopic examination: The pineal gland (epiphysis cerebri) of adult New Zealand rabbits was a fusiform-like mass surrounded by the pia-matter dorsally and bordered by the thalamus ventrally. It was composed of two portions; stroma and parenchyma. The parenchyma consisted of cortex externally and medulla internally. The latter was contained pinealocytes and astrocytes (Fig. 1a). These pinealocytes were arranged in the form of clusters or rosettes separated by numerous nerve fibers, blood capillaries and supported by astrocytes (Fig. 1b).

Two types of pinealocytes were observed within the parenchyma of the pineal gland; Pinealocytes type I and II. Pinealocytes type I were more numerous, larger in size with large rounded vesicular nuclei, clear cytoplasm and long cytoplasmic processes. While, pinealocytes type II were fewer in number, smaller in size with oval central nuclei and densely stained cytoplasm (Fig. 1c and d).

Electron microscopic examination: Electron microscopic observation of the pineal parenchyma revealed an intimate cell-to-cell association between pinealocytes and astrocytes (Fig. 2a). The astrocyte was small in size containing irregular electron dense oval nucleus and little cytoplasm. On the other hand, the pinealocytes were of two types, type I and II. The type I was larger in size with multiple cytoplasmic processes containing euchromatic large rounded centrally-situated nucleus with irregular outline and prominent electron dense nucleolus. The cytoplasm appeared electron lucent, contained numerous mitochondria, many dilated cisternae of rER, well developed Golgi apparatus, numerous free ribosomes as well as few electron dense secretory granules (Fig. 2b). The type II appeared small in size contained euchromatic oval nucleus with irregular outline and little condensed cytoplasm (Fig. 2c).

Identification

Non-specific reactions: Treatment of the prepared sections with Grimelius silver impregnation showed strong argyrophilic reaction indicated by brownish black colored-cytoplasm (Fig. 3a). By using lead hematoxylin stain, the cytoplasm of pinealocytes showed moderate to strong blue-black reaction (Fig. 3b). On the other hand, these cells reacted moderately to OFG method by the appearance of red colored cytoplasmic granules in both cytoplasm and nerve fibers (Fig. 3c).

Specific reactions: The cytoplasmic granules of the pinealocytes showed moderate reaction (purple violet color) when stained by Gomori's aldehyde fuchsin stain (Fig. 3d). In a section stained with the aldehyde thionine stain, the pinealocytes gave moderate reaction of light blue color (Fig. 3e). By using Gomori's chrome alum hematoxylin stain, the cytoplasmic granules showed strong reaction. They stained dark blackish blue color (Fig. 3f). The cytoplasmic granules of pinealocytes reacted positively with the performic acid alcian blue stain given intense blue color (Fig. 4a).

Immunohistochemically, the pinealocytes were subjected to anti-CgA antibody using PAP technique giving positive reaction, their cytoplasmic granules stained yellowish brown (Fig. 4b). On the other hand, application of PAP technique using anti-NSE antibody on the pinealocytes gave a strong reaction indicated by dark brown color (Fig. 4c).

Highly specific reaction: The application of PAP technique on the prepared sections using anti-Melatonin antibody revealed positive reaction in the form of brownish cytoplasmic granules (Fig. 4d).

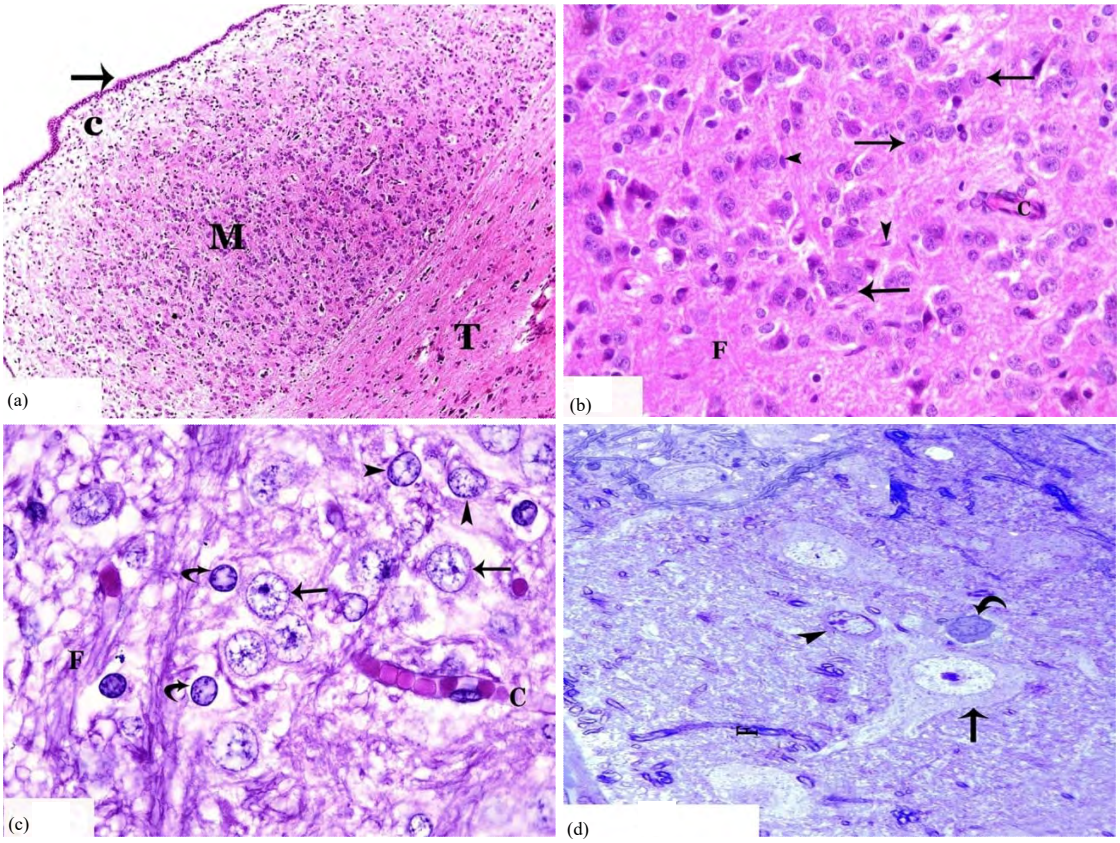

Fig. 1(a-d): A sagittal section of the pineal gland of the adult New Zealand rabbit showed, (a) A fusiform structure covered dorsally by the pia matter (arrow) and bordered ventrally by the thalamus (T). Their parenchyma consisted of cortex (C) and medulla (M) (H and E stain, X100), (b) Clusters and rosettes distribution of the pinealocytes (arrows), separated by nerve fibers (F), blood capillaries (C) and astrocytes (arrowheads) (H and E stain, X400), (c) A higher magnification of figure (1B) showing two types of pinealocytes; type I (arrows) and II (arrowheads), separated by the nerve fibers (F) and astrocytes (wrapped arrows) (H and E stain, X1000) and (d) Pinealocytes type I (arrow) and II (arrowhead) separated by nerve fibers (F) and astrocytes (wrapped arrow) (Toluidine blue stain, X1000)

DISCUSSION

The current study aimed to design a histological scheme used for identification and characterization of neuroendocrine cells (NECs) and applied this scheme on pineal gland of New Zealand rabbits as a model. This work revealed that the two types of pinealocytes were contained electron dense secretory granules. These granules were reacted positively with the applied general, specific and highly specific reactions of neurosecretory cells. These results were in accordance with that observed by Revel *et al.*[6], Regodon *et al.*[13], Arendt and Skene[14], Maronde and Stehle[15], De Carvalho *et al.*[16] and Ross and Pawlina[17].

Generally, the NECs were neither an ordinary neurons nor an endocrine cells but a combination of both. Production of a visible secretory material marks the NSCs as a gland cell[18]. The diffuse neuroendocrine system includes neurons having characters of both neurons and endocrine glands including; neural origin, expression of specific biomarkers (neuropeptides such as chromogranins, synaptophysin) and neuropeptides processing enzymes as well as presence of dense-core secretory granules[17]. Neurosecretion efficiency was a basic property that enables scientists to differentiate neurons and neurosecretory cells[19].

All established methods used for identification of NECs depended mainly upon the presence of certain amine and peptide residues produced by the NECs and there was no single technique will demonstrate all NECs[9]. From this point, the current study tried to establish a histological scheme used to characterize and identify neuroendocrine cells on the base of using general

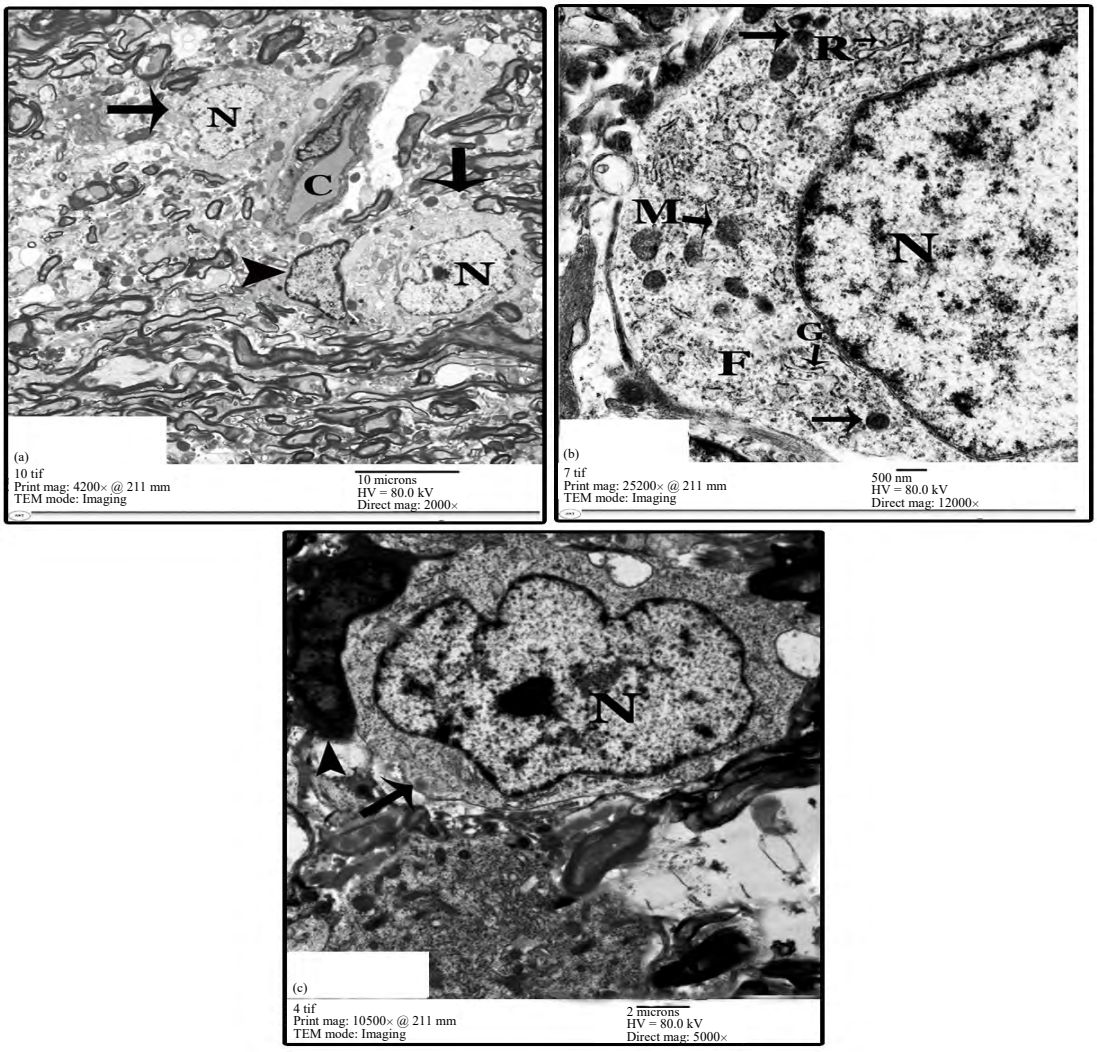

Fig. 2(a-c): An electron micrograph of the pineal gland of the adult New Zealand rabbit showing, (a) Pinealocytes type I (arrows) with euchromatic nucleus (N) and electron lucent cytoplasm. Note, blood capillary (C) and astrocyte (arrowhead), (b) A higher magnification of pinealocyte type I showing euchromatic nucleus (N) and electron lucent cytoplasm containing many mitochondria (M), rER (R), Golgi apparatus (G), free ribosomes (F) and some secretory granules (arrows) and (c) Pinealocyte type II (arrow) appeared small with oval nucleus (N) and little cytoplasm. Note presence of an astrocyte (arrowhead). Uranyl acetate and lead citrate stain; X2000, 12000 and 5000, respectively

stains that detected the general histological features of the tissues under investigation, non-specific stains were used for detection of all endocrine granules, specific reactions were used for demonstration of neuroendocrine granules and lastly application of highly specific reactions that were applied for detection of specific hormones. The pineal gland represented a part of the neuroendocrine-reproductive axis[4].

Grimelius silver impregnation technique and lead haematoxylin stain were used for detection the presence of endocrine granules. The Grimelius stain was an argyrophilic technique depends upon reduction of the bounded silver salts from Ag+1 to metallic silver Ag leading to its deposition and absorption by the cellular organelles giving positive brown color. On the other hand, Lead Haematoxylin stain was considered one of the empirical techniques used for demonstration of the endocrine granules in different body tissues[9], so authors used this stain as a non-specific stain for preliminary identification. The positive reaction to such stain was explained as the released carboxyl groups from the polypeptide secretion inside the endocrine cells able to react with and change the color of used basic dye lead

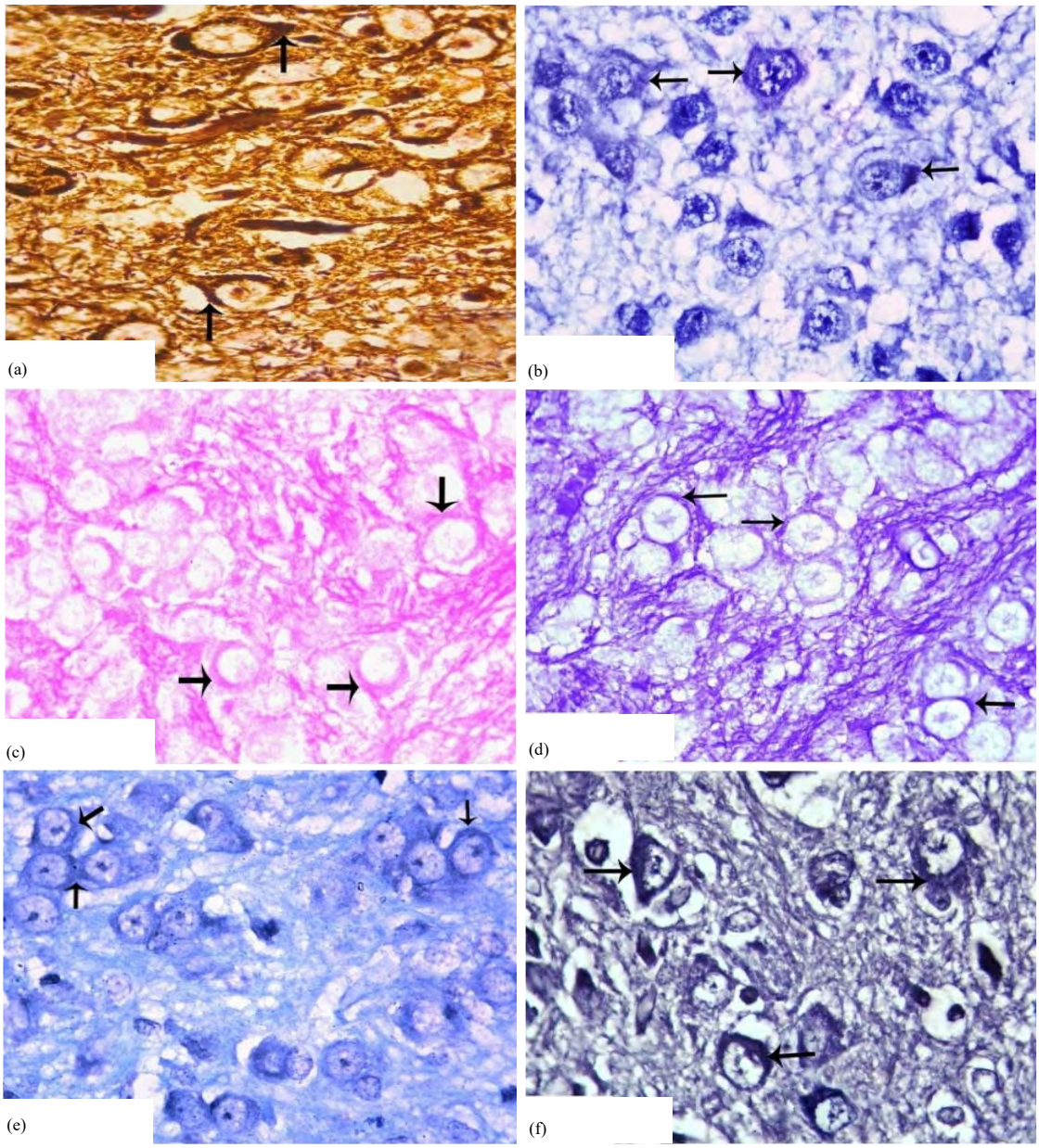

Fig. 3(a-f): A section through the pineal gland of the adult New Zealand rabbit showing, (a) Pinealocytes with strong argyrophilic granules of brownish black color (arrows). Grimelius silver impregnation stain, X1000, (b) Moderate to strong positive blue-black granules (arrows) within the cytoplasm of pinealocytes. Lead Hematoxylin stain, X1000, (c) Red colored cytoplasmic granules (arrows). OFG method, X1000, (d) Moderate reaction (purple violet color) in the cytoplasmic granules (arrows) of pinealocytes, Gomori's aldehyde fuchsin stain, X1000, (e) Moderate thionine reaction (light blue color) in the pinealocytes (arrows). Aldehyde thionine stain, X1000 and (f) Strong blackish blue granules (arrows) within the pinealocytes. Gomori's chrome alum Hematoxylin stain, X1000

haematoxylin[9,20]. The positive reaction of the pinealocytes to these stains indicated that, these cells contained endocrine granules.

Regarding using the specific stains in the present investigation, some histochemical methods were applied to demonstrate and identify the neuroendocrine granules in addition to, some immunohistochemical reactions which enable scientists to recognize the general biomarkers specific to NECs. These reactions were depending upon presence of certain amino acids inside the neurosecretory granules that

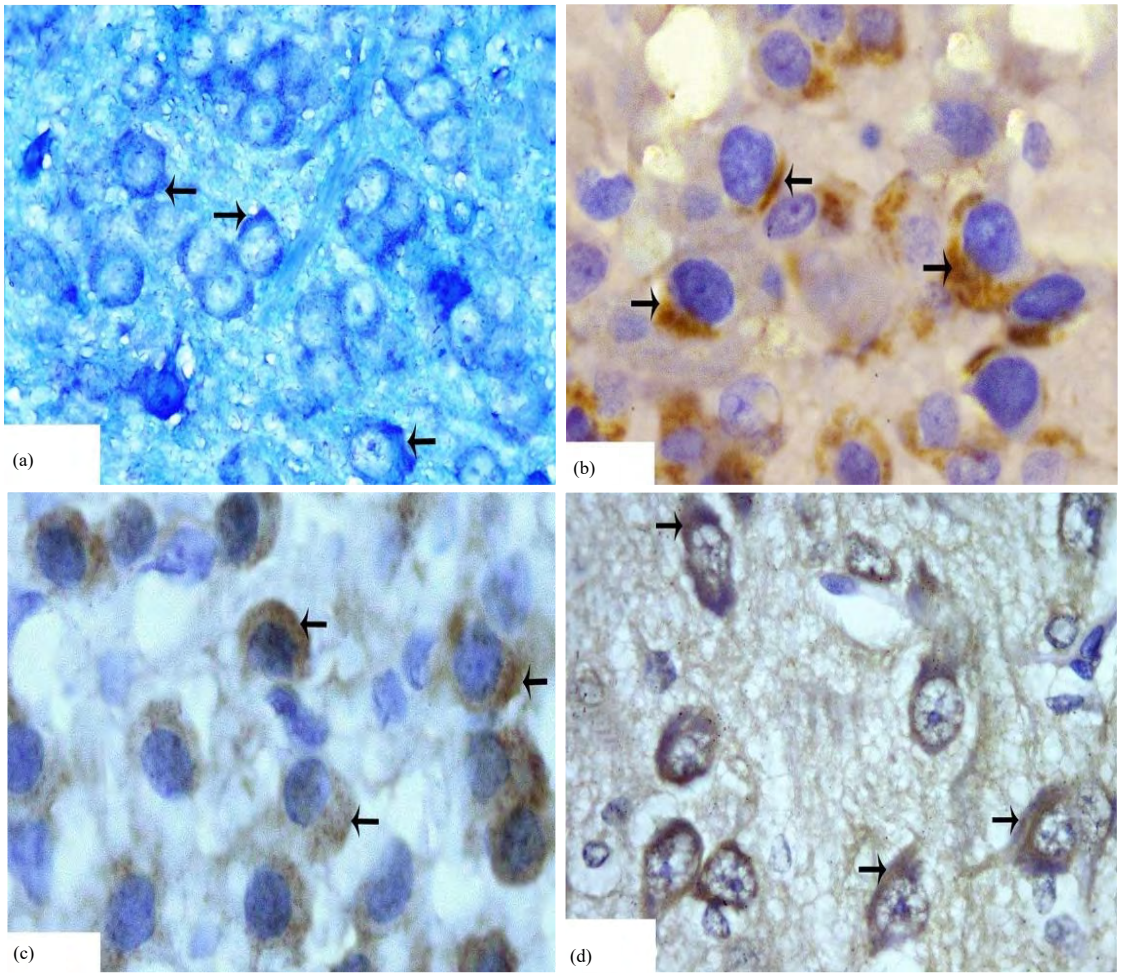

Fig. 4(a-d): A section through the pineal gland of the adult New Zealand rabbit showing, (a) Pinealocytes with intense positive blue colored cytoplasmic granules (arrows). Performic acid alcian blue stain, X1000, (b) Positive reaction (yellowish brown color) in the cytoplasmic granules of pinealocytes (arrows), PAP technique using anti-CGA antibody, X1000, (c) Strong reaction of dark brown color in the cytoplasmic granules (arrows) of pinealocytes. PAP technique using anti-NSE antibody, X1000 and (d) Positive brown color in the cytoplasmic granules of pinealocytes (arrows), PAP technique using anti-Melatonin antibody, X1000

reacted with the active radical of the used stains or by detection of resulted end products from either reduction or oxidation of the disulphide bonds[21]. The cytochemical analysis revealed positive reactions of NECs for proteins specifically for sulfur-containing groups[22]. The AF was seemed to be most excellent technique for demonstrating the neurosecretory materials[23]. This stain had strong affinity for the reducing groups of sulfated amino acids of the Van Dyke protein that present in the neurosecretory materials[24]. The NECs were rich in cystine amino acids containing a highly reducing essential groups (disulphide (-SS) which reduced to sulphydryle (-SH) groups and reacted with the active radical of the stain resulted in positive color[25]. Aforementioned explanation of positive reaction of AF stain was augmented by Mahon and Nair[26]. Performic acid alcian blue (PA-AB) stain detected the presence of disulphide (–SS) groups[27]. The performic acid oxidized the cysteine or cystine amino acids in the tissues into cysteic acid (sulphonate active acid radicals), which reacted with the active basic radical of the stain and gave the positive blue color[27].

The CgA was a member of family glycoprotein conjugates with the matrix of neurosecretory granules[28] which may regulates the production of specific peptides[29]. CgA was an immunohistochemical marker used to identify the neurosecretory cells containing numerous neurosecretory granules[9,30]. Consequently, the pineal gland under study was subjected to anti-CgA antibody using PAP technique that

reacted with an epitope on the C-terminal half of the CgA molecule and gave positive brown colored immunoreactive cytoplasmic granules. These results had been explained by Huttner *et al.*[31], who reported that the NECs synthesis a wide variety of bioactive peptides and amines that stored in their large dense-core vesicles or in small neurotransmitter synaptic-like vesicles. Some proteins were connected with these vesicles; Granins (Chromogranin and Secretogranin) and Synaptophysin which could be utilized as specific biomarkers of NECs. The presence of enolase isoenzyme inside the NECs was independent on the number of NSGs[7]. The treated sections of pineal gland with anti-NSE antibody using PAP technique showed strong dark brown immunoreactive cytoplasmic granules indicated presence of enolase isoenzyme inside the pinealocytes.

Melatonin was the output of the pineal gland. Its synthesis and release showed prominent diurnal rhythm with the peak at night[14]. The mammalian melatonin rhythm was endogenously driven by the hypothalamic suprachiasmatic nucleus and controlled by light acting via the retinohypothalamic tract and retina[32]. Application of anti-melatonin antibody using PAP technique on the pinealocytes of pineal gland of the adult New Zealand rabbit gave positive brown colored granules. Wiener[33] reported that, the pineal gland is the only part of the brain that contained so much serotonin and capable of making melatonin.

The pineal gland under investigation showed positive results to all applied reactions indicates that the pinealocytes are neuroendocrine cells. Authors recommended all histologists to apply the suggested scheme for demonstration the histological features of other neuroendocrine cells and other tissues thought to have neuroendocrine characters. In addition, further studies should be focused on such type of schemes for achievement a rapid progression in this field.

CONCLUSION

The present study introduced a preliminary design of a histological scheme used for identification and characterization of all neurosecretory cells distributed throughout the body using the pineal gland as a model.

SIGNIFICANCE STATEMENT

This study discovered a model design for histological scheme that can be beneficial for biologists especially histologists. This study will help the researcher to uncover the critical areas of determination the optimal reactions for identification and characterization of the neurosecretory cells that many researchers were not able to explore. Thus a new theory on development of other advanced methods for characterization of neurosecretory products may be arrived at.

REFERENCES

1. Frohman, L.A., 2009. Neuroendocrinology. Encyclopedia Neurosci. 10.1016/B978-008045046-9.01184-0.
2. Tessmar-Raible, K., 2007. The evolution of neurosecretory centers in bilaterian forebrains: Insights from protostomes. Seminars Cell Dev. Biol., 18: 492-501.
3. Pearse, A.G.E., 1986. The diffuse neuroendocrine system: Peptides, amines, placodes and the APUD theory. Progr. Brain Res., 68: 25-31.
4. Vollrath, L., 1981. The Pineal Organ. In: Handbuch der Mikroskopischen Anatomie des Menschen, Oksche, A. and L. Vollrath (Eds.). Vol. 7, Springr Verlag, New York.
5. Schroder, H., A. Bendig, D. Dahl, U. Groschel-Stewart and L. Vollrath, 1990. Neuronal markers in the rodent pineal gland-an immunohistochemical investigation. Histochemistry, 94: 309-314.
6. Revel, F.G., M. Masson-Pevet, P. Pevet, J.D. Mikkelsen and V. Simonneaux, 2009. Melatonin controls seasonal breeding by a network of hypothalamic targets. Neuroendocrinology, 90: 1-14.
7. Schmechel, D., P.J. Marangos and M. Brightman, 1978. Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature, 276: 834-836.
8. Bancroft, J.D. and A. Stevens, 1996. Theory and Practice of Histological Techniques. 4th Edn., Churchill-Livingstone, London.
9. Bancroft, J.D. and M. Gamble, 2008. Theory and Practice of Histological Techniques. 6th Edn., Elsevier Health Sciences, Philadelphia, PA., ISBN-13: 9780443102790, Pages: 725.
10. Hayat, M.A., 1986. Basic Techniques for Transmission Electron Microscopy. Academic Press, New York.
11. Richardson, K.C., L. Jarett and E.H. Finke, 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol., 35: 313-323.
12. Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208-212.
13. Regodon, S., J. Masot, A. Franco and E. Redondo, 2001. Postnatal maturation of parenchymal cell types in sheep pineal gland: An ultrastructural and immuno-electron-microscopic study. Rev. Med. Vet., 152: 325-333.
14. Arendt, J. and D.J. Skene, 2005. Melatonin as a chronobiotic. Sleep Med. Rev., 9: 25-39.
15. Maronde, E. and J.H. Stehle, 2007. The mammalian pineal gland: Known facts, unknown facets. Trends Endocrinol. Metab., 18: 142-149.

16. De Carvalho, A.F., C.E. Ambrosio, M.A. Miglino, C.A.F. Mancanares and F.J.H. Blazquez, 2009. Macro-microscopical aspects of the buffalo (*Bubalus bubalis* Linnaeus, 1758) pineal gland. Biotemas, 22: 127-135.
17. Ross, M.H. and W. Pawlina, 2015. Histology: A Text and Atlas: With Correlated Cell and Molecular Biology. 7th Edn., Lippincott Williams and Wilkins, Paltimore, Maryland.
18. Scharrer, B., 1967. The neurosecretory neuron in neuroendocrine regulatory mechanisms. Am. Zool., 7: 161-169.
19. Malosio, M.L., R. Benfante, G. Racchetti, B. Borgonovo, P. Rosa and J. Meldolesi, 1999. Neurosecretory cells without neurosecretion: Evidence of an independently regulated trait of the cell phenotype. J. Physiol., 520: 43-52.
20. Solcia, E., G. Vassallo and C. Capella, 1968. Selective staining of endocrine cells by basic dyes after acid hydrolysis. Stain Technol., 43: 257-263.
21. Watkins, W.B., 1975. Immunohistochemical demonstration of neurophysin in the hypothalamoneurohypophysial system. Int. Rev. Cytol., 41: 241-284.
22. Gabe, M., 1966. Neurosecretion. Pergamon Press, Oxford, pp: 373.
23. Kivalo, E. and S. Talanti, 1957. The foetal development of the hypothalamic-hypophysial neurosecretory system of the cow embryo. Eur. J. Endocrinol., 26: 471-476.
24. Sloper, J.C., 1955. Hypothalamic neurosecretion in the dog and cat, with particular reference to the identification of neurosecretory material with posterior lobe hormone. J. Anat., 89: 301-316.
25. Scott, H.R. and B.P. Clayton, 1953. A comparison of the staining affinities of aldehyde-fuchsin and the Schiff reagent. J. Histochem. Cytochem., 1: 336-352.
26. Mahon, D.C. and K.K. Nair, 1975. Stoichiometry of the aldehyde fuchsin staining reaction for proteins. J. Histochem. Cytochem., 23: 652-659.
27. Adams, C.W. and J.C. Sloper, 1956. The hypothalamic elaboration of posterior pituitary principles in man, the rat and dog; histochemical evidence derived from a performic acid-Alcian blue reaction for cystine. J. Endocrinol., 13: 221-228.
28. Lassmann, H., C. Hagn, R. Fischer-Colbrie and H. Winkler, 1986. Presence of chromogranin A, B and C in bovine endocrine and nervous tissues: A comparative immunohistochemical study. Histochem. J., 18: 380-386.
29. Wand, G.S., M. Takiyyuddin, D.T. O'Connor and M.A. Levine, 1991. A proposed role for chromogranin A as a glucocorticoid-responsive autocrine inhibitor of proopiomelanocortin secretion. Endocrinology, 128: 1345-1351.
30. Pearse, A.G., 1977. The diffuse neuroendocrine system and the apud concept: Related "endocrine" peptides in brain, intestine, pituitary, placenta and anuran cutaneous glands. Med. Biol., 55: 115-125.
31. Huttner, W.B., H.H. Gerdes and P. Rosa, 1991. The granin-(chromogranin/secretogranin) family. Trends Biochem. Sci., 16: 27-30.
32. Ganguly, S., S.L. Coon and D.C. Klein, 2002. Control of melatonin synthesis in the mammalian pineal gland: The critical role of serotonin acetylation. Cell Tissue Res., 309: 127-137.
33. Wiener, H., 1968. External chemical messengers. IV. Pineal gland. N. Y. State J. Med., 68: 912-938.