



Apoptotic Features in Normal Rabbit's Adenohypophysis Cells (Electron Microscopic and Quantitative Study)

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Abstract : The earliest notable signs of the apoptosis such as the chromatin mass condensation and gradually migration peripherally toward the nuclear envelope were recognized. The terminal end of the apoptotic pathway, represented with apoptotic bodies and membrane blebs or blisters appearances recognized too. In general, apoptotic cells were decreased with progressing animal age; however, there was only significant increase of the apoptotic cells between baby group and adult group in rabbit adenohypophysis with t-value 2.46 and $p < 0.05$.

Key words: Apoptotic cells, Adenohypophysis, Rabbit, Electron Microscope.

أشكال الذوي في خلايا النخامية الغدية للأرنب (دراسة بالمجهر الإلكتروني ودراسة كمية)

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الخلاصة: لقد تم إستعمال المجهر الإلكتروني النافذ في الدراسة الحالية لغرض التعرف على الخصائص النوعية والمظهرية (الفحص البايولوجي) لخلايا النخامية الغدية للأرنب كوسيلة مساعدة للتحري عن أشكال الذوي على صعيد النواة والخلية فضلاً عن التحليل الإحصائي للخلايا الذاوية

طبقاً لعمر الحيوان. وتمت ملاحظة بعض العلامات المبكرة لآلية الذوي في الخلايا وهو تكاثف الكروماتين وهجرته بشكل تدريجي محيطياً باتجاه الغلاف النووي. إن العلامات النهائية والختامية لمسار الذوي والمتمثلة بظهور الأجسام الداوية وبثرات غشائية قد أشر أيضاً. وتمت ملاحظة نقصان في عدد الخلايا الداوية مع تقدم عمر الحيوان، وعلى كل حال، كان هناك إرتفاع معنوي وحيد للخلايا الداوية لمجموعة الرضع عند مقارنتها بمجموعة البالغين.

Introduction

The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multi cellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions (1).

During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues (2). Taken together, apoptotic processes are of widespread biological significance, being involved in e.g. development, differentiation, proliferation/homoeostasis, regulation and function of the immune system and in the removal of defect and therefore harmful cells (3).

Due to its importance in such various biological processes, programmed cell death is a widespread phenomenon, occurring in all kinds of metazoans(4) such as in mammals, insects (5), nematodes (6), and cnidarians (7).

Molecular mechanisms of apoptosis signalling pathways involve the caspases proteins family, extrinsic apoptosis pathway as well as mitochondria as central regulators of intrinsic apoptosis pathways(8). The aim of this study was to find qualitative morphological characteristics for rabbit adenohipophysis as an adjuvant investigation of the apoptotic nuclear and cellular features as well as statistical analysis of them according to their age.

Materials and Methods

Animals and Pituitary Specimens

A total of 6 healthy rabbits were used in the present study. Animals were classified into three groups according to their maturity period: baby, young, adult (9) and sacrificed by decapitation under ether anesthesia. The skulls were opened and the brains were removed while the pituitary glands were dissected out with their sella turcica and sphenoid bone. Another microscopic dissecting process was used to remove sella turcica and sphenoid bone from fixed pituitary bodies.

Conventional Electron Microscopy

The adenohipophysis (lateral wings) of 6 pituitary glands were dissected and fixed in 2% glutaraldehyde for 24 hours. The material was cut into small pieces (1x1mm), another fixation in 1% osmium tetroxide and embedded in araldite. Thick sections (about 1 μ m) were stained with methylene blue and observed by light microscopy in order to select fields. Ultrathin sections were mounted on cooper grid, stained with uranyl acetate and lead citrate, and examined with transmission electron microscopy at 80 kV (10).

Statistical Analysis

Statistical evaluation was performed by t-test for multiple comparisons. Data are reported as mean \pm SEM (10).

Results and Discussion

The earliest notable signs of the apoptosis are the chromatin mass which were condensed gradually and migrated

peripherally toward the nuclear envelope while the other organelles were preserved, the notable mitochondria, secretary granules, vesicles, ribosomes and even the exocytosis process were notably Figure(1); this finding was in agreement with Wyllie *et al.*(10) and Harbor (11).

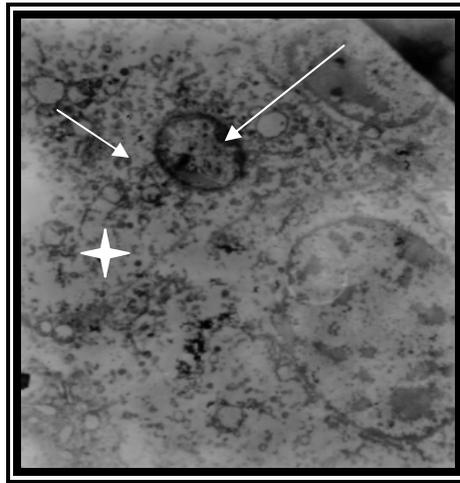


Figure (1):Electron micrograph of pro-apoptotic cell in rabbit pituitary gland showing the emigration of condensed chromatin towards nuclear envelope(arrow) with existence of mitochondria (star) and other cellular components (7900 x).

At the way of developing (apoptotic process), the nucleolus dispersed with extensive peripheral chromatin condensation which were sharply delineated uniform against the nuclear

envelope, these events associated with discontinuous nuclear envelope Figure(2), this result was similar to that obtained by Al-Sheakh (12).

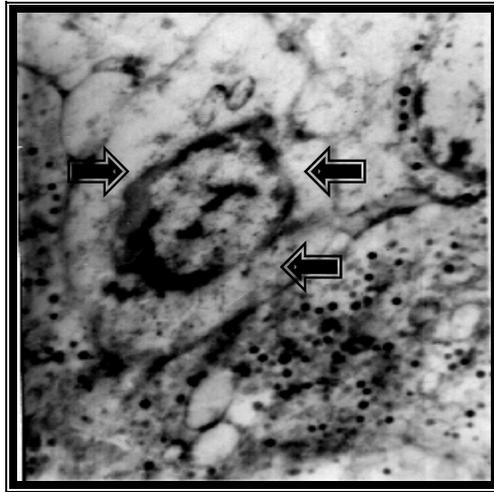


Figure (2):Electron micrograph of apoptotic cell in rabbit pituitary gland showing the discontinuous of nuclear envelope, black arrow (5800 x).

The chromatin condensation considered as the most recognizable characteristic features of the apoptotic cells, the nuclear chromatin would be compact and aggregated to form a dense, amorphous and osmiophilic mass Wyllie

et al.(13), following the cytoplasm shrinkage after the cleavage of lamins and actin filaments Figure (3) which occurred under plenty of an exogenous factors (14).

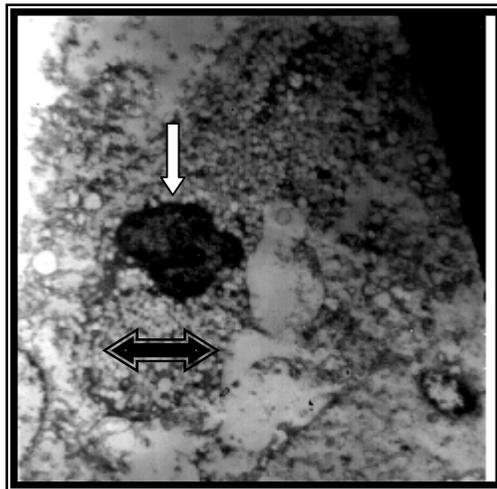


Figure (3):Electron micrograph of apoptotic cell in rabbit pituitary gland showing the compacted chromatin (white arrow) and cytoplasm shrinkage (black arrow) (3400 x).

At the terminal end of the apoptotic pathway, the apoptotic cell shows a small dark granular material which represents the apoptotic bodies Figure(4), moreover,

the membrane blebs or blisters Figure (5) which often appeared towards the end of the apoptotic process.

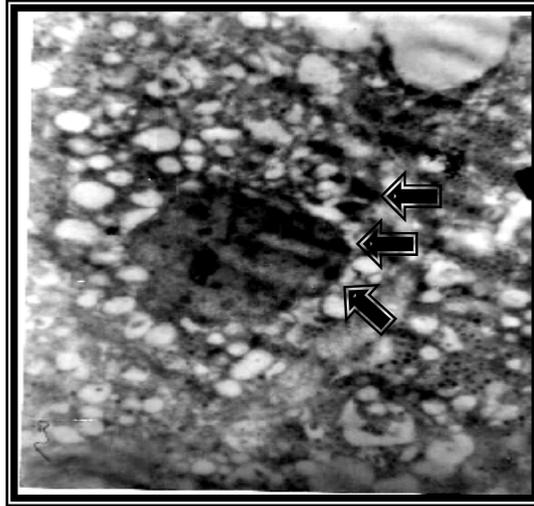


Figure (4):Electron micrograph of apoptotic cell in rabbit pituitary gland showing the apoptotic bodies, black arrow (4600 x).



Figure (5):Electron micrograph of apoptotic cell in rabbit pituitary gland showing the cytoplasmic blebs, black arrow (4600 x).

Finally, the phagocytic cells are responsible for removing apoptotic cells from the tissue in a clean tidy fashion after packaging themselves into a form that allows for easy clearance by

macrophages Figure(6) which promoted through the translocation of phosphatidylserine from the inner leaflet to the outer surface (15).

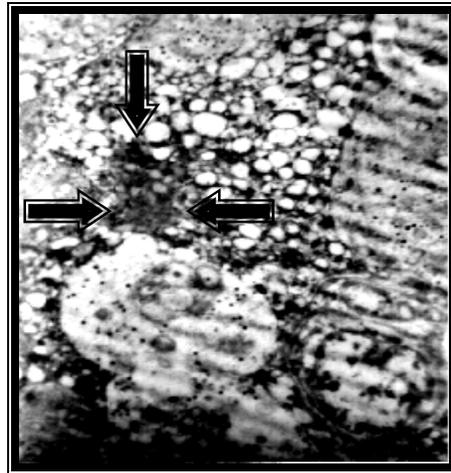


Figure (6): Electron micrograph of apoptotic cell in rabbit pituitary gland showing easy clearance of apoptotic bodies by the macrophages, black arrow (5800 x).

In order to demonstrate the comparison between different apoptotic age groups within the rabbit pituitary gland, there was a significant increase in R1 apoptotic cells compared with R3 with t-value 2.46 and $p < 0.05$; in contrast, there was no significant increase in the mean \pm S. E. between R1 and R2

apoptotic cells with t-value 1.43 as well as between R2 and R3 with t-value 1.36 and $p > 0.05$ table(1). These findings corresponded with Holmes and Ball (16), and partially with Salman (17), this may due to her studying on male mice under physiological conditions.

Table (1): Comparison of the apoptotic cells between different groups of age within the rabbit pituitary gland.

Animal group	Mean \pm S.E.	t-value
R1	15.9 \pm 1.55	1.43
R2	12.6 \pm 0.75	
R2	12.6 \pm 0.75	1.36
R3	10.0 \pm 0.85	
R1	15.9 \pm 1.55	2.46*
R3	10.0 \pm 0.85	

Note: The abbreviations of R1, R2 and R3 indicate an animal group according to their age as a baby, young and adult respectively in the rabbit.

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