Morphometric study on the Ag-NOR changes in skeletal muscle resident cells with aging

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Abstract

Background: Aging is the deterioration of mature organism resulting from time dependent irreversible changes. The effects of aging on skeletal muscle cells have not been much-elucidated using Ag-NOR analysis.

Objectives: This study aims to demonstrate the effects of aging on Ag-NOR in morphometric & counting aspects.

Materials and methods: The Extensor digitorum longus muscle of forty Albino male rats with age ranging from 27 days up 18 month were studied. Paraffin blocks were performed & sectioned. Ag –Nor stained sections were de-waxed, rehydrted, developer solution was used. Morphometric Analysis of the Ag-NOR stained nuclei through using a soft wear GLI (Global lab image 2) with a microscope connected to PC Unit, a software used to analyze the picture that seen through the microscope, nuclear area, nuclear perimeter, and roundness were calculated,& counting of the number of Ag-NOR stained nucleoli per stained cells.

Results: In neonate age group, the nuclei have high affinity to the stain. High proportion of nuclei can be recognized, with the higher count of Ag-NORs per cells.

- In adult age groups the affinity to the stain is reduced, the nucleus appears to have smaller count of Ag-NORs per cells.
- In old age group the staining intensity seem to be highly reduced, the nucleus seem to have single, rounded Ag-NOR.

Conclusion: A significance differences is seen in Ag NOR_s in cells of skeletal muscle fibers with aging demonstrated by counting and morphometric method of Ag-NOR analysis.

Key words: Skeletal muscle, Ag NOR. Morphometry.

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Introduction

Aging is defined as the deterioration of mature organism resulting from time dependent irreversible changes in all members of species, such that with the passage of time they became increasingly unable to cope with the stress of the environment, and their increasing the probability of death [(1)]. Recent theory of aging based on telomere uncapping at senescence [(2).]

Two aspects of muscle cell phenotype may be affected by aging:

1. The proliferative capacity.
2. The ability to differentiate [(3)].

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Nucleolar organizer regions (NORs) are loops of DNA, which contain ribosomal RNA genes. They are transcripted by RNA polymerase I, and are of vital importance for ultimate synthesis of protein. Ag-NORs are acidic proteins associated to the NORs, which are selectively stained by silver colloid technique [(4)].

Ag-NORs are related to the proliferative capacity, reflecting essentially cell cycle speed. The increase in their number would signify a shorter time of cell cycle [(5)].

The number of NOR at any given stage of cell cycle appears to be inversely proportional to the cell cycle time, thus the higher the amount of NOR, the shorter the cycle time [(6)], and the faster the rapidity of cell proliferation as recognized by (Brugal in 1994) [(7)].

However, the effects of aging on skeletal muscles have not been much-elucidated morphometricly. This study

aims to demonstrate the effects of aging on Ag-NOR in morphometric aspects.

Materials and methods

Animal housing and sampling:

A sample of forty Albino rats (Rattus norvegicus) of male rats was selected for this study, their age ranging from (27-day) up to (18-month).

They were divided into three age groups:

A. 1st age group (Neonate: 1st month of life).

B. 2nd age group (Adult animals: 6-12 month old).

C. 3rd age group (Old animals: More than 12-month old).

Sacrificed animal were killed with chloroform .Extensor digitorum longus muscle was selected for the study, it was taken out by cutting it from it's both ends, and divided transversely into two halves and put into a fixative [8].

General histological preparation of paraffin blocks:

Fixation done by using fisher fixative for 12 hours sections then Paraffin blocks were prepared [9] & sectioned at (5-6µM) thickness using Richert –Jung 3030 –mot Biocut microtome.

Ag –Nor (Argyrophilic nucleolar organizer region):

Sections were stained with Ag-Nor according to Ploton *et.al* [10].

Morphometric Analysis of the Ag-NOR stained nuclei:

Through using a soft wear GLI (Global lab image 2) in which a microscope is connected to PC Unit, and a software is used that analyze the picture that is seen through the microscope ,and captured to be stored in the digital memory. Displayed by the monitor, and analyzed by the software options. The nuclear area, nuclear perimeter, and roundness were calculated through encircling the nucleus that are stained with Ag-NOR by the marker through using the mouse of the computer, and set the software options, the results were expressed in an excel work sheet. Morphometric analysis of the Ag-NOR stained area had been studied by Lorand- & Carvalho 1998 [11] and was considered a valuable index for the nuclear activity yet, perimeter and roundness coefficient were used for the first time in this study.

Counting of Ag-NOR stained nucleoli:

Considering the enumeration of each silver stained dot per cell directly at the microscope focusing through the section thickness at very high magnification (100X) according to the recommendation of Crocker *et., al* 1989 [12].

Results

All examined sections stained with silver nitrate Ag-NOR stain in this study show adequate staining, but variation in the staining intensity among the different age groups was noticed. In neonate age group, the nuclei have high affinity to the stain ,they stained darkly brown, and the muscle fibers stained light brown, high proportion of nuclei can be recognized per section (Figure 1) the nucleus itself seem to have multiple Ag-NOR stained nucleoli that are small sized, rounded ,solitary, darkly stained structures, can be seen through focusing at the site of the nucleus at a high magnification (Figure4, Table 2) the morphometric analysis of the mean nuclear area show the largest value in neonate, the mean nuclear perimeter also is very high, and the nuclei are nearly rounded in shape since mean roundness value is high (as the value of

roundness approach 1 the structure is nearer to a circle) (Table 1) .

In adult age groups the affinity to the stain is reduced, the nucleus appear as single dot, small proportion of the nuclei can be recognized per section with reduction in the number of Ag-NORs per cell (Figure 2 , Table 2) the mean nuclear area, mean nuclear perimeters are also reduced, and the nucleus is nearly oval rather than rounded .

In old age group the staining intensity seem to be highly reduced, the nuclei are lightly stained ,also the muscle fibers, and smaller proportion of nuclei that can be recognized per section (Figure 3), the nucleus seem to have single ,solitary, rounded Ag-NOR, that can be seen through careful focusing at the site of the nucleus at a high magnification (Figure 5, Table 2) the nuclear area, mean nuclear perimeters are greatly reduced in old age group, and nuclear roundness value is very small (Table 1).

Discussion

In this study, all sections showed adequate Ag-NOR silver staining, the muscle fibers are stained in a light brown color and muscle fiber cells, satellite cells are stained with dark brown color.

The satellite cells considered as the active cell in skeletal muscles and as Ag-NOR staining depends on the transcriptional activity of the cells [(14)]. More recently it was suggested that the amounts of silver stained proteins are related to cell proliferation activity [(15)], so the more rapidly growing the tissue or (cells) , the more Ag-NOR proteins are present [(16)]. While the myonuclei are not stained with Ag-NOR since they are mitoticly inactive [(17).]

Ag-NOR technique has been used to asses the degree of differentiation, since clusters of Ag-NORs are only observed in proliferating cells, and Ag-NOR number decreases with cell maturation [(11)].

The argyrophilia of the nucleolar Ag-NOR is a good cytochemical marker of rRNA and of the level of its transcription, consequently the Ag-NOR stainability give further information on the actual or potentially active substructure of the nucleolus, and allow study of their number [(10)]. Their number, size and distribution within the nucleoli at the level of light might be expected to reflect cellular activity, and proliferation [(18)].

In neonate age group, large proportion of nuclei that stained with Ag-NOR can be seen in the section (Figure 1), and these nuclei posses the larger nuclear area, since the Ag-nor stained nuclear area has a strong linear correlation with the speed of cell proliferation [(19)].

In neonatal age group, it was observed that nuclei had a larger perimeter, and they are nearly rounded in shape. The nucleus posses several small, solitary, rounded to oval nucleoli (Figure 4, Table 2), this can be due to a high proliferation rate in neonate age group ,where there is a higher dispersion of the silver precipitations due to an impairment of their aggregation into nucleoli, giving rise to many small nucleoli as mentioned by (Crocker& Hotstadter) [(12,19)].

In adult age group there is a marked reduction in the amount of nuclei that stained with Ag-NOR in the section(Figure 2), accompanied by a reduction in the Ag-NOR stained nucleoli per cell (Table 2), and in nuclear area, perimeter. Roundness of nuclei is nearly rounded in shape (Table 1).

In old age group there is only single Ag-NOR stained nucleus in the section (Figure3, Table 2). A reduction in the Ag-NOR numbers was noticed with terminal cellular differentiation as

in a leukemia cell line, and with aging of stimulated lymphocytes [20 & 21]. This accompanied with lower value of nuclear area and perimeter, the nucleus of old age group posses only a single nucleolus (Figure 5), as Ag-NOR decreased with cellular maturation [16 & 22].

In old age group the marked reduction in the number of Ag-NOR staining nuclei in the section, due to a reduction in the number of satellite cells and the Ag-NOR stained structure might represent the reserve of satellite cells, where even in the absence of transcription or proliferation there is a basal level for Ag-NOR proteins [23].

Table 1:The values of morphometric analysis of mean area, perimeter, and roundness of Ag-NOR stained nuclei in the four age groups.

Parameters	Neonate	Adult	Old
Mean Area (micron)^2	**8.890629**	**2.90035**	**1.82137**
Mean Perimeter (micron)	**13.71641**	**8.306701**	**7.462056**
***Mean Roundness**	**0.662692**	**0.411671**	**0.331703**

*** Roundness:** a parameter of comparison of nuclear shape, as its value approaching (1), the nucleus is nearly circular, otherwise, it's oval, spindle shape, or other shapes.

****** All measures were done on oil immersion but pictures here on different magnifications for presentation purposes.

Table 2:The total number of Ag-NOR stained nucleoli per cell in the different sections for each age group.

Sections	Neonate	Adult	Old
Section 1	12	5	1
Section 2	12	5	1
Section 3	13	5	1
Section 4	11	6	1
Section 5	10	7	1

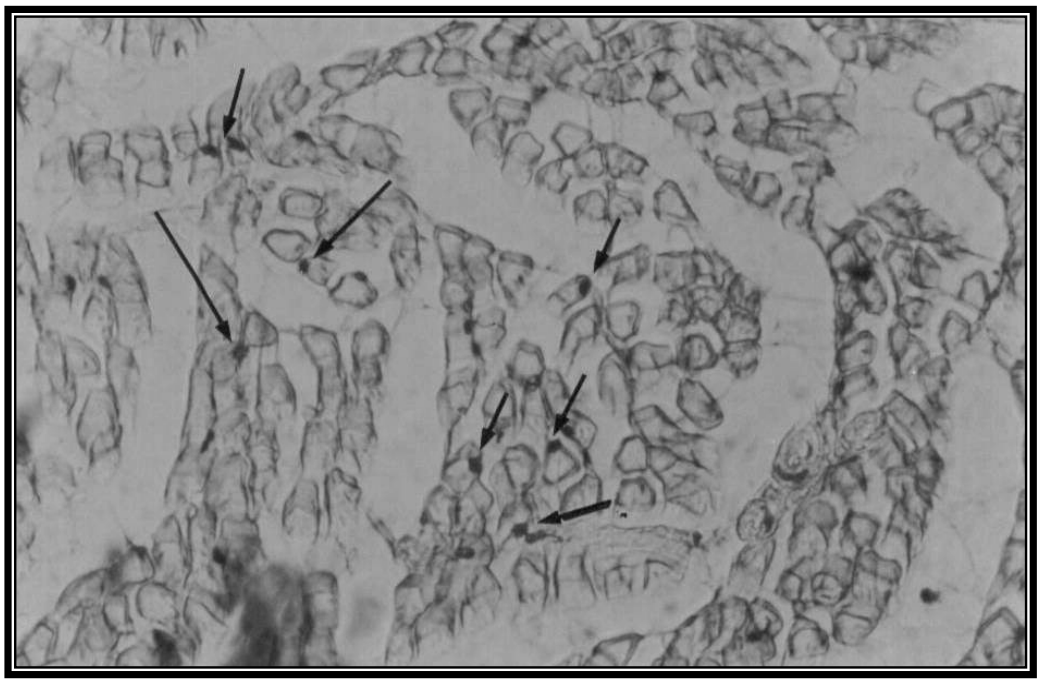

Figure 1: Cross section in skeletal muscle of neonate age group, with high number of nuclei that stained with Ag-NOR silver nitrate stain, the (arrows) demarcate the site of nuclei that have high affinity to the stain. Ag-NOR silver nitrate stain(X800).

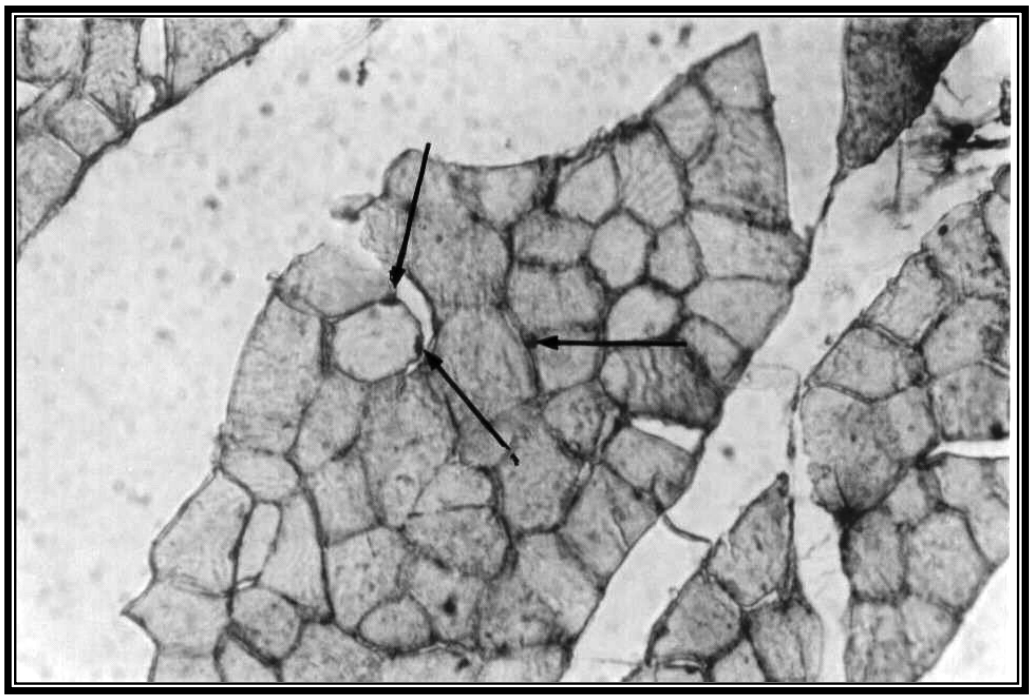

Figure 2: Cross section in skeletal muscle of adult age group, with reduced number of nuclei that stained with Ag-NOR silver nitrate stain (arrows), Ag-NOR silver nitrate stain (X800).

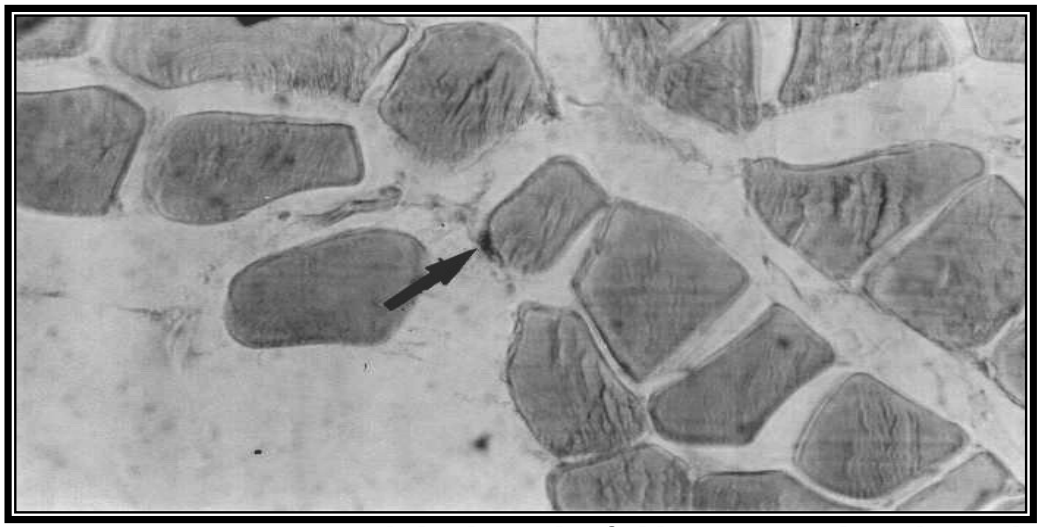

Figure 3: Cross section in skeletal muscle of old age group, show the single nucleus that stained with Ag-NOR silver nitrate stain (arrow), Ag-NOR silver nitrate stain, (X800).

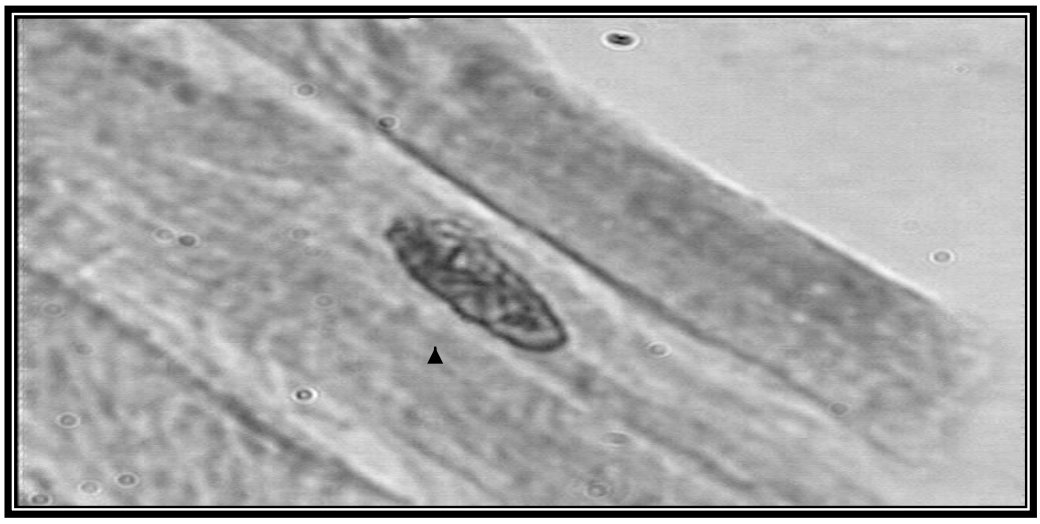

Figure 4: Neonate nucleus with multiple Ag-NORs, Ag-NOR silver nitrate stain, (X2000).

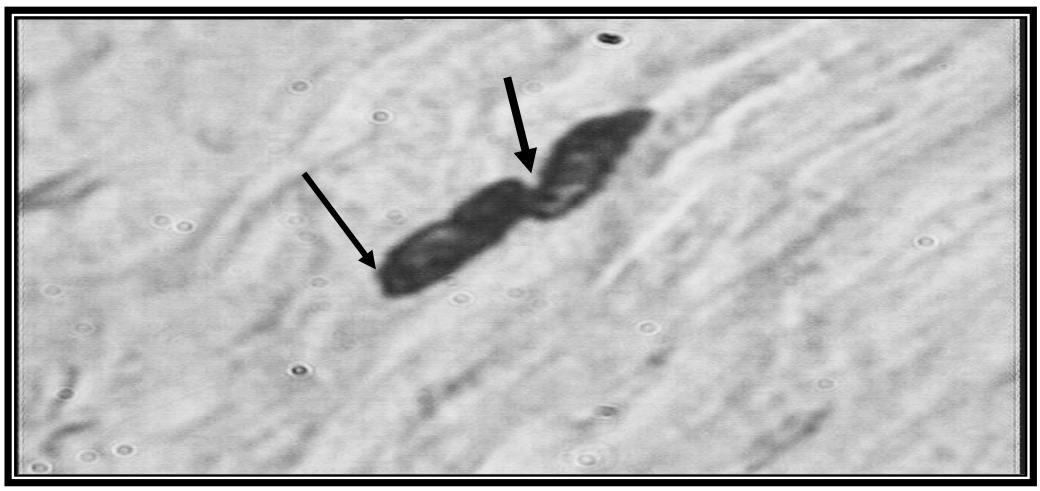

Figure 5: Old age group, show single solitary Ag-NOR per a darkly stained nucleus, Ag-NOR silver nitrate stains (X 2000).

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