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RESEARCH ARTICLE

Age-related alterations in the lacrimal gland of adult albino rat: A light and electron microscopic study

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ABSTRACT

Background: Age related changes in the lacrimal gland are associated with alterations in the structural organization and functional response in the gland of diverse mammalian species. Dry eye syndrome is one of the most common ocular problems in the world especially in old age. It results when the lacrimal gland fails to secrete proteins and fluid in sufficient quantity or appropriate composition.
Aim of the work: The present study is designed to demonstrate the influence of aging on the structure of the lacrimal gland of albino rat and to provide a morphological basis to explain the pathogenesis of the dry eye syndrome with ageing. It also aims to carry out a comparative analysis of age-dependent changes in male and female rats and to address how the lacrimal gland ages in each sex.
Material and Methods: Eighty albino rats were used in this study. The animals were divided into two age groups, young adult and senile. Tear secretion was measured using a modified Schirmer test. Corneal impression cytology of the anesthetized rats was done. The glands were subjected to gross morphologic examination, microscopic examination using H&E, PAS, Masson's trichrome and Giemsa stains. Electron microscopic examination was done in addition to quantitative histomorphometric estimations included acinar density, ductal count and mast cell count.
Results: Light microscopic examination of the lacimal glands of the senile rats revealed different pathological changes. These included acinar, ductal as well as stromal changes. Electron microscope examination of the lacrimal gland of the senile group showed a decrease in the electron dense secretory vesicles, mitochondrial swelling and lipofuscin-like inclusions were frequently seen in the cytoplasm of acinar cells in senile rats.
Conclusion: The structural changes in the lacrimal glands of senile rats were associated with reduction in tear secretion as well as alterations in corneal epithelium. Gender difference in lacrimal gland structure was recorded.

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1. Introduction

Age related changes in the lacrimal gland are associated with alterations in the structural organization and functional response in the gland of diverse mammalian species (van Haeringen, 1997; Draper et al., 1999). It was reported that aging impairs tear secretion and induces changes in the lacrimal gland structure and function as well as ocular surface properties (Tsubota, 2007).

Tear film covering the epithelium of the exposed surfaces of the cornea and conjunctiva has been described as a trilaminar structure, consisting mainly of a watery aqueous layer, overlying a thin mucous layer, with a superficial lipid layer (Butovich et al., 2008; Standring et al., 2008).

The lacrimal gland is the primary source of the aqueous portion of the tear film (Dartt, 2004; Standring et al., 2008), therefore, it plays a crucial role in maintaining a healthy environment for the actively functioning epithelium of the cornea and conjunctiva (Schechter et al., 2010). Dry eye syndrome is one of the most common ocular problems in the world, especially in old age (Kim et al., 2009). It results when the lacrimal gland fails to secrete proteins and fluid in sufficient quantity or appropriate composition (Kanski and Bowling, 2011). It was reported that female sex and androgen deficiency are risk factors for dry eye disease (Lemp et al., 2007). Therefore, gender differences were taken into account in the present study of the aging lacrimal gland.

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The present study is designed to demonstrate the influence of aging on the structure of the lacrimal gland of albino rats and to provide a morphological basis to explain the pathogenesis of the dry eye syndrome with ageing. This study also aims to carry out a comparative analysis of age-dependent changes in male and female rats and to address how the lacrimal gland ages in each sex.

2. Material and methods

2.1. Animals

Eighty albino rats of Wistar strain (40 males and 40 non pregnant females) from Research Institute of Ophthalmology were used in this study. The animals were divided into two age groups as follows:

Group I (young adult): This group consisted of 40 animals of three to five months age (Sullivan et al., 1990). It was further subdivided into two subgroups:

- Subgroup I A: Consisted of 20 male rats.
- Subgroup I B: Consisted of 20 female rats.

Group II (senile): Consisted of 40 animals of 17-20 months of age (Ricciardi et al., 2002). It was further subdivided into two subgroups:

- Subgroup II A: Consisted of 20 male rats.
- Subgroup II B: Consisted of 20 female rats.

In the present study, the guidelines on use research animals were followed. All animals were fed a constant adequate nutrition diet and allowed free access to drinking water ad libitum. All the rats were sacrificed at noon after at least two weeks of environmental adaptation. Diseased rats were excluded.

2.2. Methods

The animals were anesthetized by intraperitoneal injection of ketamine (5 mg/100 g body weight) and xylazine (2 mg/100 g body weight). They were weighed and the obtained data recorded. The lacrimal glands have been shown to exhibit side specific dimorphism (Ricciardi et al., 2002), therefore, only the gland on the left side was used in this study. Tear secretion was measured in the left eye of the rats in both groups using a modified Schirmer test with one mm width and 20 mm long strip of filter paper placed in the conjunctival fornix of the eye for five minutes. The length of the wet part was measured, recorded and statistically analyzed (Batista et al., 2012).

For impression cytology, corneal epithelial cells were collected from the anesthetized rats by swabbing the temporal area of the left cornea with filter paper. The filter paper was then fixed with 70% ethanol glacial ascetic acid and formalin, stained with periodic acid-Schiff (PAS) and hematoxylin, then transferred to slides and examined under a light microscope (Batista et al., 2012). The rats were then sacrificed with an overdose of an intraperitoneal anesthetic. An incision was made between the eye and the ear on the left side and the exorbital lacrimal gland was quickly extracted. The surrounding connective tissue was removed. The glands were subjected to:

(A) Gross Morphologic Study: The absolute weight of each gland was measured using an electric balance and the relative gland weight was calculated as a percentage of the lacrimal gland weight divided by the total body weight. The glands were grossly examined by the naked eye or using a magnifying lens. Glands with abnormal appearance (size, color and lobulation) were excluded to avoid any congenital abnormality or any diseases affecting it.

(B) Light Microscopic Examination: Half of the gland was fixed immediately in 10% formalin saline for 24 h. The specimens were then washed and dehydrated in ascending grades of ethanol (70%, 90% and100%). They were cleared in xylene for two hours. Impregnation and embedding were done in soft paraffin wax at 45–50 °C for three hours and in hard paraffin at 60 °C for one hour. Six μm thick semi-serial sections at every eleventh section of the whole gland were prepared for Hematoxylin and Eosin, PAS, Masson Trichrom, Giemsa's stain

(C) Electron Microscopic Examination: The other half of the gland was prepared for ultrastructural examination using transmission electron microscope (TEM). The sections were examined and photographed by JEOL JEM 1010 transmission electron microscope in electron microscope research laboratory, Faculty of Agriculture Research Center.

(D) Quantitative Morphometric Study: All quantitative morphometric estimations were done on the previously stained histologic sections of lacrimal glands using image analyzer (Leica Imaging System Ltd., Cambridge, England). Images were captured live on the screen from sections under a light microscope (Olympus Bx-40, Olympus Optical Co. Ltd., Japan) with an affixed video camera (Panasonic color CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan). The video images were digitized using "Lecia Qwin 500C" which is a Leica's windows based image analysis tool kit fitted to an IBM compatible personal computer with a color monitor. Quantitative morphometric study included:

(1) Morphometric Study in Hx & E stained sections:

Acinar density: Acinar density was calculated by counting the acini in central fields under magnification 400. Acinar density measurement was performed on 12 randomly chosen fields from each gender in both young adult and senile groups (two fields/animal in six animals/age/gender).

Acinar area: Acinar area is defined as quantitation of acinar size. Area measurement was performed on 90 randomly chosen acini from each gender in both age groups under magnification 400 (15 acini/animal in six animals/age/gender).

Ductal count: Ductal count was calculated after counting each type of duct in central fields under magnification 200. Counts were performed on 12 randomly chosen fields from each gender in both age groups (two fields/animal in six animals/age/gender).

Ductal diameters and wall thickness: The outer and inner diameters as well as wall thickness were measured in central fields under magnification 200. Measurement was done on the widest diameter of the transversely cut ducts of 60 randomly chosen ducts from each gender in both age groups (10 ducts/animal in six animals/age/gender).

Lymphocytic foci count: One lymphocytic focus was defined as consisting of 50 or more cells (Rios et al., 2005). Lymphocytic foci were counted in central fields under magnification 100 in 12 randomly chosen fields from each gender in both age groups (two fields/animal in six animals/age/gender).

(2) The percentage area of collagen fibers in Masson's Trichrome stained sections: The image analyzer was first automatically calibrated to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Using the measuring field menu; the area, area percentage and standard measuring frame of a standard area were chosen. In each chosen field, lacrimal gland tissue was enclosed inside the standard measuring frame and then the connective tissue area was masked by a blue binary color to be measured. The measurements were done under magnification 400 in 12 randomly chosen fields from each gender

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