Legal Medicine 17 (2015) 437–442

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Morphological and histological changes in eye lens: Possible application for estimating postmortem interval

Gemma Prieto-Bonete, Maria D. Perez-Carceles *, Aurelio Luna

Department of Legal and Forensic Medicine, Biomedical Research Institute (IMIB-Arrixaca), Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Spain

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 3 September 2015
Accepted 14 September 2015
Available online 14 September 2015

Keywords:
Postmortem interval
Eye lens
Corneal transparency
Proteins
Histology

ABSTRACT

Establishing the postmortem interval is a very complex problem in Forensic Science despite the existence of several macro- and microscopic methods. In the case of ocular methods, most are based on an evaluation of the biochemical components of the vitreous humour 24–36 h after death, but, to our knowledge, there are no studies on the relationship between lens and the postmortem interval. Since the lens is protected between the vitreous humour and the aqueous humour inside the eyeball, postmortem changes are assumed to start later in the lens. To evaluate the usefulness of using the lens to establish the postmortem interval, we examined 80 rabbit lens enucleated 24, 48, 72 and 96 h after death, assessing changes in sphericity and absorbance at different wavelengths and any histological alterations. Both sphericity and absorbance were seen to decrease to a statistically significant extent, and there was a gradual loss of structure and organisation of the lens components as a function of the postmortem interval. Modifications in the lens were seen to be useful for determining the postmortem interval between 24 and 96 h.

1. Introduction

Establishing the postmortem interval continues to be one of the most complex problems in Forensic Science. Studies based on ocular data to establish PMI are few and tend to be based on an evaluation of the biochemical components of the vitreous humour, such as sodium, potassium, chloride, lactate and hypoxanthine 24–36 h after death [1–4].

The lens is positioned in the eye behind the iris. The anterior lens surface is bathed by aqueous humor, while the posterior lens surface is in contact with the vitreous body [5]. Contains 1000–3000 layers of fiber cells [6]. The adult lens contains two kinds of fiber cells: (i) those located in the cortex (the outermost layers of the lens), which are not yet mature and still contain organelles (including mitochondria), all of which are degraded through protease- and nuclease-regulated processes, leaving behind membrane-enclosed bags of crystallines, and (ii) those located in the nucleus (the core of the lens), which are mature and do not contain organelles [6]. Into subcellular organelle evacuation during maturation is necessary to ensure the transparency of the lens, as organelles scatter light, whereas ordered proteins (crystallins) do not. Protein synthesis and protein degradation are minimal or non-existent, and crystallins and perhaps other proteins that were synthesized at the birth of the cell persist throughout the life of the organism [7].

The transparency of the eye lens thus depends on the regular alignment of elongated fiber cells, which perform the difficult task of stacking together neatly to fill a spheroidal volume, filled with cytoplasmic crystallins and cytoskeletal intermediate filaments encased in membranes made from a few integral membrane proteins. The lens proteins belong to common protein families, but the lens tends to have its own unusual version [8].

Crystallines, which are expressed as three different isoforms (α-crystallin, β-crystallin and γ-crystallin), are major cytoplasmic components of the vertebrate eye lens that constitute >90% of the total protein content in eye lens fiber cells and >35% of their wet weight [7]. The chaperone action of α-crystallin is vital for maintaining eye lens transparency.

The reasons for using rabbit as an experimental model to study the lens are the following: First, both the rabbit and human lens have branched sutures, although the former is of the "line" type and the latter of the "star" type. For this reason, the rabbit lens can be considered as a simplification of the more complex organisation of the fibers of the human eye [9–13]. Secondly, the rabbit lens is closer in size and sphericity to the human lens than other

* Corresponding author at: Department of Legal and Forensic Medicine, Biomedical Research Institute (IMIB-Arrixaca), Regional Campus of International Excellence "Campus Mare Nostrum", School of Medicine, University of Murcia, E-30100, Spain.
E-mail address: mdperez@um.es (M.D. Perez-Carceles).

http://dx.doi.org/10.1016/j.legalmed.2015.09.002
1344-6223/

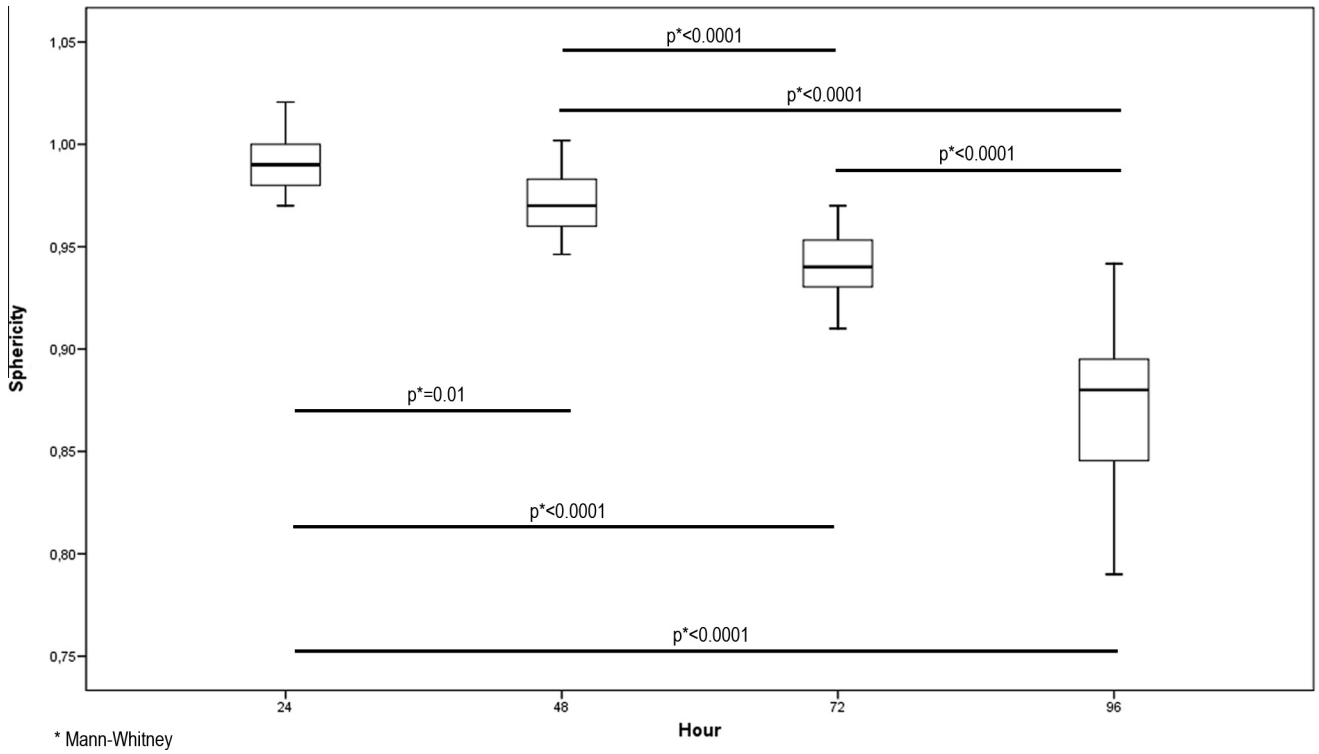

Fig. 1. Sphericity of the lenses and statistically significant differences between right and left eyes for postmortem intervals.

lens commonly used in experiments, such as mouse or rat. The first study to use rabbit eye as an experimental model measured the thickness of the cornea by ultrasounds [2]. Lastly, in this study what we have achieved of the functional parameters of rabbit lens provides a basis for comparison with the human lens [10,14–16].

While there are many studies on the morphological and histological changes related to certain pathologies, we have found no reference to the use of the lens to establish the PMI. Determining the changes in lens transparency postmortem would be a useful starting point for testing the possibility of determining visual impairment conditions' in a cadaver [17]. The postmortem evaluation of the lens would provide the added benefit of associating different forms and stages of cataracts with accidents, thus affording new information relevant to the development of preventive measures [18].

Apart from estimating the PMI, the age of the subject could be ascertained between 24 and 48 h after death by applying radiocarbon techniques to the lens [17]. In conclusion, the postmortem determination of lens opacity would provide helpful information that could be used during legal proceedings and it would be also a good complement to clinical data, and fundamental in cases where there is no medical documentation.

The aim of the present study was to assess whether the postmortem morphological and histological modifications that take place in the lens may be related to the postmortem interval itself.

2. Materials and methods

2.1. Sample collection

The lens ($n = 80$) were taken from 40 rabbits with an average age of 84.02 days old (75–95 days old) sacrificed in a local meat-processing company [19]. All the animals used were treated in the normal way and were not killed for the sake of the experiment described. In the laboratory of the Forensic Medicine Department of the University of Murcia (Spain), the lens were left exposed to the air in a room with a mean temperature of 21.3 °C at 24 hpm, 21.4 °C at 48 hpm, 22.4 °C at 72 hpm and 22.7 °C at 96 hpm.

The animals were cared for following Spanish law (RD 1201/05) according to the principles of EU directive EU 86/609. This study was approved by the Ethics Committee of the University of Murcia (Spain).

2.2. Enucleation and measurements of sphericity and absorbance

The 80 lens were classified into four groups of 20 samples each. Every 24 h all the lens from a given group were enucleated (first group 24 h postmortem, second group 48 hpm, third group 72 hpm and the last group 96 hpm) by making a lateral incision and cutting the orbital muscles [20]. Once extracted, the 20 lens were placed in physiological saline, the their absorbance was measured at 365, 370, 375, 415 and 420 nm using a Shimadzu UV-160 spectrophotometer [21]. The absorbance of each eye lens was measured directly in a 1-cm plastic cuvette designed and developed in our laboratory, and an empty cuvette was used as blank. The special design of the cuvette allowed the lens to be held in place vertically, so that its own weight and the effect of gravity did not affect its structure since it was supported by the edges, leaving the central part in its correct anatomical position when light rays were directed at it. After measuring, the lenses were photographed using a Nikon DX digital camera (AF-S DX NIKKOR), at 20 cm distance and with a resolution of 10.2 megapixels. The image analysis program image tool UTHSCSA was used to measure the greatest and smallest diameter, and to calculate the sphericity of the lens. In this way, we could assess any modification in transparency or translucence as a function of the PMI.

2.3. Histological study

After visual examination by image tool, the eyes lens were fixed in 10% buffered formalin for 22–30 days and embedded in paraffin. Sections (4 nm thick) were obtained from each paraffin block and placed on SuperFrost (Menzel-Gläser, Braunschweig, Spain) plus