CORRELATION OF BOTH POTASSIUM LEVEL IN VITREOUS HUMOR AND HISTOPATHOLOGICAL CHANGES IN RAT'S CORNEA TO TIME SINCE DEATH

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ABSTRACT

Introduction: Estimation of time since death remains an important issue in forensic investigations and the eye has a great value in this field. **Objective:** This work was designed to prove the Correlation of both potassium level in vitreous humor and histopathological changes in rat's cornea to time since death. **Material and Methods:** Sixty adult male albino rats were divided equally into 2 main groups. Group 1 was subdivided into six groups according to known postmortem intervals (0, 24, 48, 72, 96 & 120 hrs). It was used for chemical estimation of potassium level in vitreous humor at each time interval. Only the clear samples of vitreous humor were taken after exclusion of any injured or diseased eye. Group 2 was subdivided into six groups at the same postmortem intervals and used for detection of histopathological changes in rat's cornea by both light microscope (Toluidine blue stain) and scanning electron microscope (SEM). **Results:** As regard biochemical results, vitreous humor samples showed significant time dependent increase in the potassium level up to 120 hours postmortem. Regarding histopathological results, light microscopical examination of rat's cornea showed gradual degenerative changes from 24 hrs postmortem in the form of cloudy swelling and separation between its layers to complete necrosis of epithelial cells (only clusters of basal cells seen attached to cell membrane) at 96 hrs postmortem, while the endothelium showed slight affection at 24 hours postmortem, then subjected to gradual degenerative changes to complete necrosis and loss of its cells at 72 hours postmortem, finally, the details of the cornea were not obvious in both epithelium and endothelium at 96 hours postmortem. Scanning electron microscope (SEM), confirmed the previous results in the epithelial layers with gradual desquamation, while the endothelium is nearly normal at 24 hrs postmortem. Then, gradually increased to show loss of the endothelial cells at 72 hrs postmortem with final loss of cyto-architecture pattern in both epithelium and endothelium up to 120 hrs postmortem. **Conclusion**: potassium level in vitreous humor shows gradual time dependent increase in its level up to 120 hours. The changes in the rat's cornea are considered as good indicator of postmortem interval up to 96 hours using light and electron microscope, so, we can depend on one of these method or both of them not only for crime investigation to estimate time since death, but also in corneal donors for corneal transplantation.

Keywords: Eye, vitreous humor, potassium, cornea, desquamation, SEM and postmortem interval.

INTRODUCTION

Estimation of the time since death is an important part after postmortem examination. However, it is extremely difficult because timing of onset and the rates of postmortem changes are usually affected by unpredictable endogenous and exogenous factors [1].

In early postmortem period, which is defined as the time between the death and the onset of putrefaction, reliable results can be obtained while in later period it become more difficult and the problem worsens when body is mutilated, skeletonized or invaded by animals. Time bound histological and histo-chemical study of degenerative changes in various organs and tissues may be a good solution [2& 3]

The use of post-mortem chemistry has been one of the most important procedures for the forensic pathologist. Although blood remains the media of choice for many substances, the varying degrees of postmortem changes are making the evaluation difficult or impossible. To overcome the inadequacies of blood studies, a wide variety of other fluids are continuously investigated like CSF, pericardial fluid, synovial fluid, and vitreous humor. From all of these, vitreous humor is the most utilized medium and the most widely used method is

estimation of potassium concentration in vitreous humor [4].

The vitreous is a transparent, colorless and visco-elastic gel formed of complex network of biologic molecules mainly collagen and hyaluronic acid [5]. It is present at birth and does not change over the course of aging. The relative mouse vitreous volume is significantly smaller than the human vitreous, since the mouse lens occupies nearly 75% of its eye. This has made biological studies of mouse vitreous challenging [1]

Vitreous humor is the only fluid which is unique and preferred because it is anatomically separated, resistant for putrefaction for a long time, more sterile as no bacteria were visible in gram stained smear during postmortem intervals, up to 76-100 hrs. In addition, it is the most organs spared in homicidal cases even with multiple stab wounds. Furthermore, chemical changes occur at a slower rate than in blood and CSF [6&7].

Many studies revealed that environmental factors like humidity and temperature did not have any effect of the level of potassium in vitreous humor [8]. Also, factors like age, sex, cause of death, season of death and refrigeration of sample did not affect the vitreous humor potassium values [9].

The physical composition of vitreous humor is similar to cornea, but contain very few cells, no blood vessels, 98-99% of its volume is water (as opposed to 75% in the cornea) with salts, sugars, Vitrocin (a type of collagen) and Opticin (a type of protein) **[10].**

The typical healthy rat's cornea consists of an avascular transparent structure in an arc shape located at the foremost anterior segment of the eyeball. The typical curvature of of rat's cornea is 1,4 mm with 60μm in thickness [11].

Cornea continue to receive oxygen for metabolism, thus remain viable for a longer period than most other tissues/cells, fortunately for this reason, "eye banks" can successfully harvest cornea many hours after death and transplants can thus occur [12].

The eye is an important forensic sample in determining time passed since death through many methods. Up till now, we do not know how long epithelial and endothelial cells of cornea can survive postmortem. The aim of the present work was to determine potassium level in vitreous humor, in addition to the histopathological changes in the cornea and prove their correlation with time since death through five postmortem intervals in eyes of adult male albino rats.

MATERIALS AND METHODS

I-Animals

Sixty male albino rats weighing 150-200g were used in this study. The animals were obtained from the Experimental Animal Houses of Faculty of Veterinary Medicine, Zagazig University. All animals were left for 10 days of passive preliminaries in order to adapt themselves to their new environment. They were maintained on a standard diet and allowed water ad-libitum. All ethically approved conditions used for animal housing and handling was considered. The Institutional Review Board (IRB) of the faculty of Medicine, Zagazig University, ethically approved the study protocol.

The animals were sacrificed by an overdose of ether, and then divided equally into two main groups. Each group was divided equally into 6 subgroups (a, b, c, d, e & f) at (0, 24, 48, 72, 96 & 120 hours) (24 hours interval).

Group 1: Thirty rats were used for detection of potassium level in vitreous humor (five rats in each group were used at time of death and through the following five postmortem intervals.

Group 2: Thirty rats were used for corneal histopathological preparation. The five rats in each group were used at the time of death and through the same postmortem intervals.

The cornea cannot be taken from group 1 because the technique by which vitreous humor was taken cause marked corneal degeneration that interferes with the corneal results.

Ocular injuries and abnormalities were excluded in all rats of the present study especially group 1 because vitreous values are valid only when obtained from an intact globe [13]. Also, chronic diseases like renal failure were excluded in group 1 of this study because autolytic and metabolic postmortem processes cause postmortem increase in K+ level of

vitreous humor are affected by chronic kidney diseases [14].

II- Sampling:

(A) Group 1: Evisceration of vitreous for biochemical analysis of potassium was done through the following steps according to **Skeie et al. [15].**

1-Anterior segment dissection:

Scleral tissue posterior to the limbus is grasped with 0.22 forceps and the globe (eyeball) is stabilized. Then make a linear incision in the cornea from limbus to limbus using a microsurgical blade. A 0.12 colibri forceps is then used to grasp the cornea at the incision. The anterior chamber fluid is then absorbed with a Wick-Cel surgical spear.

2-Lens evisceration:

While grasping the cornea to stabilize the globe, a fine curved needle holder (or curved dressing forceps) is inserted behind the lens towards the posterior aspect of the globe. The needle holder is partially closed and pulled forward. Pressure is applied using the forceps on the external surface of the sclera, which pushes the lens forward through the corneal incision while leaving the eye wall intact. The vitreous appear as a translucent gel that partially adherent to the lens.

3-Retina evisceration:

Continue to stabilize the globe with 0.12 colibri forceps grasping the corneal incision. A fine curved needle holder (or curved dressing forceps) is placed as far posterior to the globe as possible, near the optic nerve. The needle holder is partially closed and pulled forward. Pressure is applied using the forceps on the external surface of the sclera, which pushes the retina forward through the corneal incision. The vitreous appears as a translucent gel adherent to the retina. The retina is visualized as a yellow, vascularized tissue. The retina -vitreous tissue is then placed into the filtered centrifuge tube containing the lens- vitreous tissue.

4-Filtered centrifusion:

The filtered centrifuge tube is placed in a benchtop centrifuge. Spin at 14,000x G for 12 minutes. Aspirate the eluent from the lower chamber, which is the vitreous. The retina remains in the upper chamber.

Samples were frozen at -70C until assayed for potassium **Madea and Rödig [16]** and the values were expressed in m Eq/l.

(B) Group 2: The eyes are enucleated at 0, 24, 48, 72,96 & 120 hrs postmortem at 18-20 c. Then eyes were opened at the limbus and the corneas were removed then fixed in 2.5 % glutraldehyde-paraformaldehyde for 6 hours and then washed in 0.1 molar phosphate buffer (PH 7.4). The intact cornea of each rat was then divided into two equal pieces, taking care not to damage the epithelium; one was evaluated by light microscope and the other was evaluated by electron microscope [17&18].

III-Biochemical study:

Potassium was determined in vitreous humor samples using commercial Kit potassium (Tubidimetric Method Biodiagnostic, Egypt). Spectrophotometer was used for potassium analysis in vitreous humor. Each sample was centrifuged at 3000 pm for 10 minuets and the supernatant fluid was taken for determination of potassium,

In the present study, vitreous humor samples were withdrawn from right eye of each eye to avoid the significant difference in K+ concentration between the two eyes of the same rat that was proved by **Pounder et al. [19].**

IV- Histopathological study:

(A) Group 1: All animals of this group were sacrificed, small parts of the kidney in each animal was fixed in formalin and stained with hematoxilin and eosin for light microscopical examination.

(B) Group 2:

1- Light microscopical examination: The 1st half of the cornea is divided into small pieces and placed in 1% osmium tetroxide in phosphate buffer. The specimens were dehydrated with increasing concentrations of ethanol and embedded in epoxy resin. Semi-thin sections were cut by ultra-tome, stained with toluidine blue and all corneal layers were examined by light microscope [20].

2- Scanning electron microscopical examination (SEM): The 2nd half was divided into two pieces by a diamond knife, placed into 1% osmium teroxide for two hours at 20°C, and then dehydrated in a grading

concentrations of ethanol to 100% and reached a critical point of drying using carbon dioxide as the transitional fluid. The pieces were mounted on small metal plates with conducting carbon cement and coated with gold before epithelium and endothelium of each specimen being examined and photographed with a Philips SEM scanning electron microscope [21].

Statistical Analysis:

Data was statistically analyzed using SPSS-10 statistical software. Numerical data was first summarized and expressed as mean and standard deviation. (ANOVA) and Post Hoc Tukey's tests was used. Difference was considered significant at $p < 0.05$.

RESULTS

(A) Biochemical results (group 1):

Analysis of data of the present study revealed a statistically significant time dependent increase in potassium concentration up to 120 hours ($P < 0.0001$) using Analysis of variance and Post Hoc Tukey's tests (Table 1), which was linear in fashion (Figure 1).

The mean potassium concentration was increasing gradually from 05.19±1.33 at zero time to reach 21.72±1.65 at 120 hrs.

(B)Histopathological results:

Group 1: All kidney samples from all rats of this group showed normal glomeruli and tubules (Fig. 2).

Group 2:

Group 2a (Zero time postmortem) (Fig.3):

The cornea of this group showed normal five layers: 1- the epithelium formed of five layers; two layers of superficial flattened epithelial cells, three layers of wing polygonal cells and a single layer of basal cuboidal cells. 2- Bowman's layer; modified anterior layer of stroma. 3- Stroma formed of collagenous lamellae running parallel to each other with keratocytes in between. 4-Descement's membrane; thick basement membrane of the endothelial cells. 5- Endothelium; single layer of cells with flattened nucleus (Fig. 3a). Scanning electron microscope examination of the epithelium showed the polygonal superficial cells in a mosaic pattern with microvilli in corneal epithelial surface. The dark and light cells of the superficial layer were clearly observed. The endothelium showed normal hexagonal appearance (Fig. 3b).

Group 2b (24 hours postmortem) (Fig. 4):

Slight degenerative changes appear in the cornea of this group, these included fluid accumulation inside the cytoplasm (Cloudy swelling) causing its vaculation. The superficial cells of the epithelium constitute the major part of the corneal epithelium with swollen nuclei. Also, all cells were flattened with loss of palisading appearance of the most basal layer. Edema of the stroma was observed. The endothelium was intact, however, some areas was thin and even rarefied (Fig. 4a). SEM examination demonstrated swollen epithelial cells with nuclear bulging. The dark and light cells of superficial layer were preserved at that time because this difference decrease as postmortem time increased. The endothelium was nearly normal, only some areas showed ill-defined borders causing loss of regular hexagonal structure (Fig. 4b).

Group 2c (48 hours postmortem) (Fig. 5):

Marked changes were showed in the cornea 48 hours postmortem in the form of disorganization of the corneal epithelium as the epithelium become thinned out with loss of cyto-architecture in the basal cell layer, rupture of keratinocyte cell membrane with fragmented or smudged nuclei (karyohexis), focal separation between the superficial and deep epithelial layers. The stroma appeared wavy and the keratocytes were abnormal. Their nuclei appeared pale, some of which showed margination of the chromatin, while others appeared pyknotic (loss of cell membrane, chromatin and cytoplasm, only dark stained nuclei). The endothelial cells appeared degenerated with vaculolated cytoplasm (Fig. 5a). Examination by SEM revealed desquamation in the epithelial cells, the endothelial cells appeared swollen with nuclear bulging (Fig. 5b).

Group 2d (72 hours postmortem) (Fig. 6):

Marked damage was observed 72 hours postmortem as the epithelium showed rarefaction with rupture of cell membrane, nuclear membrane and only remnant of nuclear debris (Karyolysis) as the superficial cell layer broke up with separation between superficial and deep epithelial layers. The stroma

appeared dense with separation between lamellae. Descement's membrane was focally separated from the stroma with complete loss of endothelial cells (Fig. 6a). SEM examination showed that all the superficial cells were desquamated, however, some of deep cells were damaged. Some of endothelial cells appeared swollen while others were lost (Fig. 6b).

Group 2e (96 hours postmortem) (Fig. 7):

Complete loss of epithelial cells in the form of sheets was observed 96 hours postmortem, only clusters of basal cells could be seen attached to cell membrane. Most of Descement's membrane is separated from stroma that showed marked separation between its lamellae. No endothelial cells were observed (Fig. 7a). SEM examination confirmed these results as loss of cyto-architecture pattern appeared in both epithelium and endothelium (Fig. 7b).

Group 2 f (120 hours postmortem) (Fig. 8):

The histopathological details of the cornea was not obvious at the level of both epithelium and endothelium layers due to marked autolytic changes, also at the level of SEM examination, it showed the same autolytic changes as (Fig.7b), so we can not depend on cornea in estimation of time since death after 4 days (96 hrs).

Table (1): Potassium concentration in vitreous humor among the different postmortem intervals (n:30).

	Group 1						
Groups n=5	**Group 1a (0 time) Mean±SD**	**Group 1b (24 hrs) Mean±SD**	**Group 1c (48 hrs) Mean±SD**	**Group 1d (72 hrs) Mean±SD**	**Group 1e (96 hrs) Mean±SD**	**Group 1f (120hrs) Mean±SD**	**P**
Mean K+ (mEq/l)	05.19 ± 1.33	08.56±1.16 a	11.95±1.65 b	15.78 ± 1.19 c	18.98±1.13 d	21.72±1.65 e	<0.001*

SD: Standard deviation, n: number, K+: potassium, *Significant
a: Significant when compared with group 1a
b: Significant when compared with group 1b
c: Significant when compared with group 1c
d: Significant when compared with group 1d
e: Significant when compared with group 1e

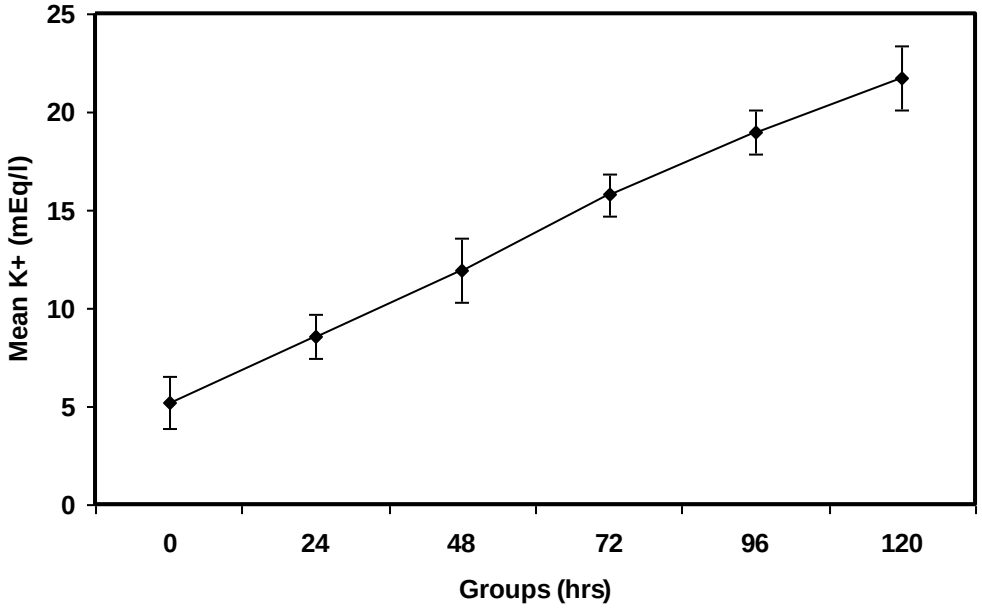

Figure (2): Vitreous humor potassium concentration (mEq/l) in all studied postmortem groups

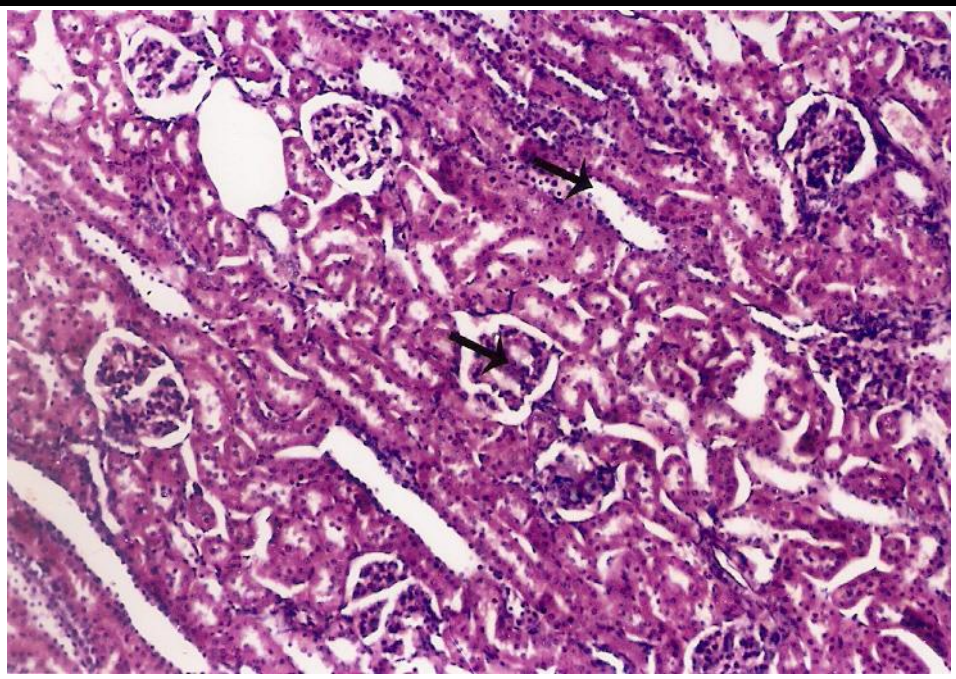

Figure (2): Normal kidney tissue with normal glomeruli and tubules.

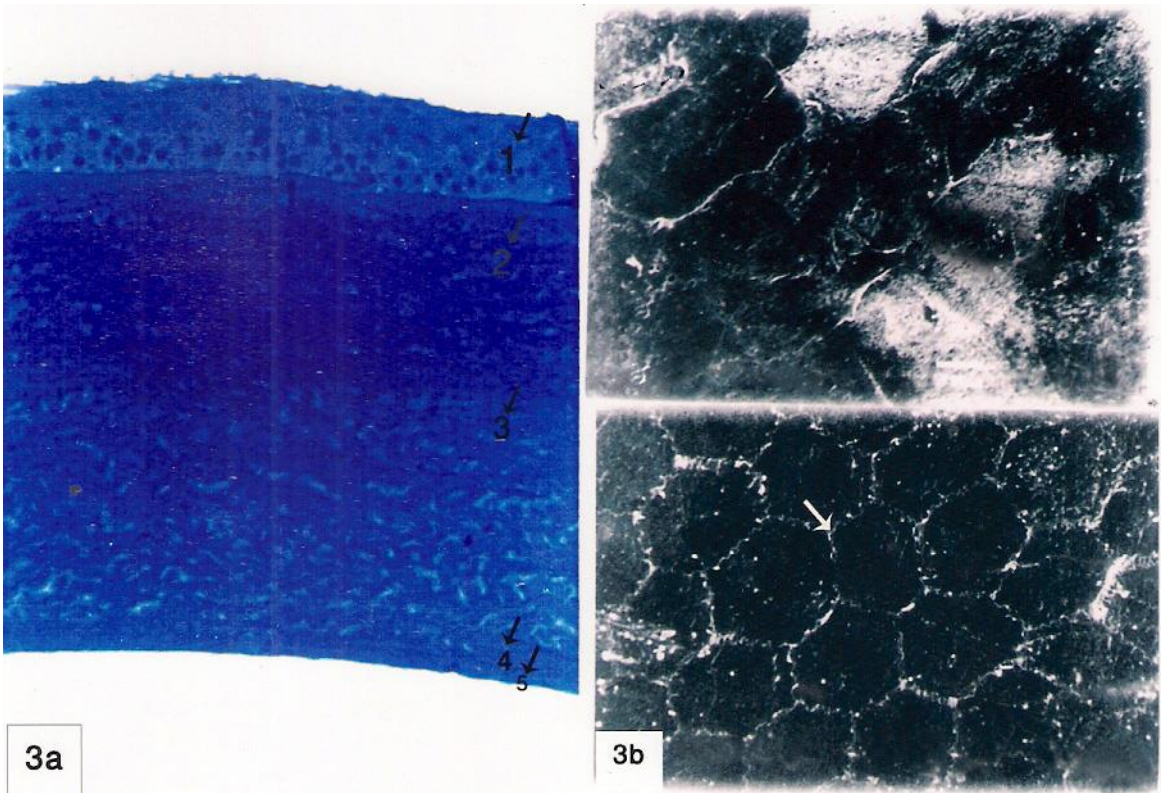

Figure (3): Zero time group (a) light micrograph of cornea showing the normal five layers of cornea (arrows) epithelium (1), Bowman's layer (2), Stroma (3), Descement's membrane (4) and Endothelium (5) (X 250). (b) scanning electron micrograph showing normal polygonal mosaic pattern with microvilli in corneal epithelial surface with dark and light cells, also, normal and regular hexagonal cell pattern of corneal endothelium (arrow) (X1000).

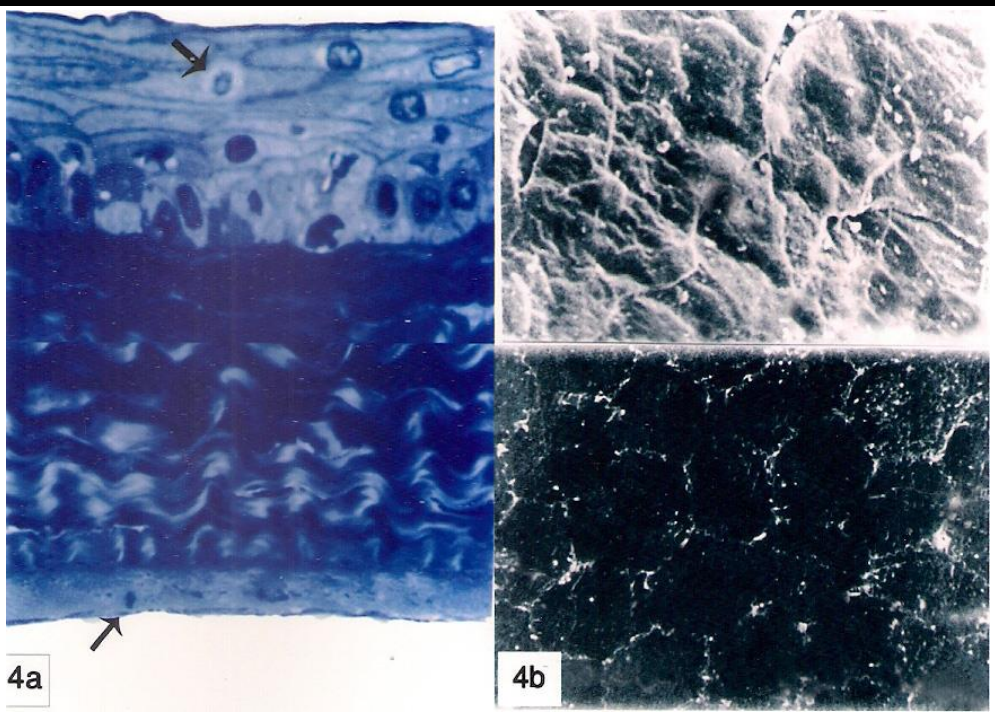

Figure (4): 24 hrs postmortem group (a) Light micrograph-showing degeneration of corneal epithelium in the form of cloudy swelling with vaculated cytoplasm (arrow 1). The superficial area constitutes the major thickness. The stroma appears edematous and the endothelium is nearly intact with slight rarefaction in some areas (arrow 2) (X1250). (b) Scanning electron micrograph showing swollen epithelial cells with nuclear bulging while the endothelial cells showed few areas of indistinguishable cell boundaries of the hexagonal pattern (arrow) (X1000).

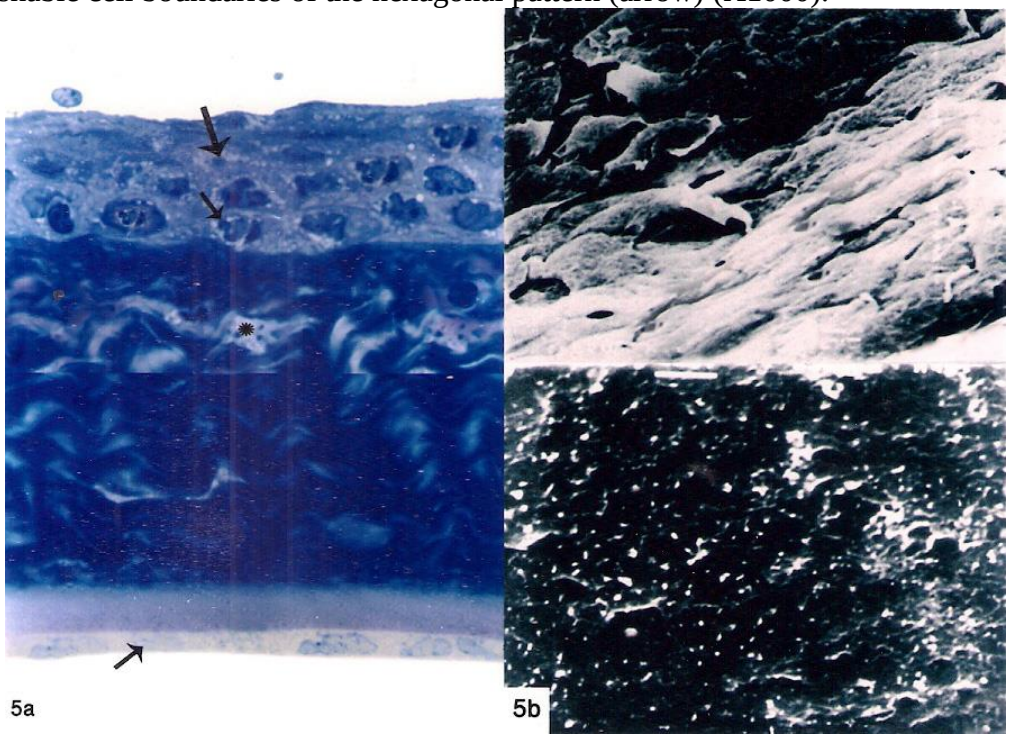

Figure (5): 48 hrs postmortem group (a) Light micrograph of cornea showing disorganization of corneal epithelium, loss of cyto-architecture of the basal cells with karyohyxis (thin arrow), other nuclei appeared pale with margination of chromatin and some showed pyknotic nuclui (thick arrow). The stroma showed edema with abnormal keratocytes (star), in addition to obvious damage of the endothelium (arrow) (X1250). (b) Scanning electron micrograph showing areas of desquamation in epithelial cells. Endothelial cells appeared irregular with nuclear bulging in some areas while few areas showed complete loss of endothelial cells. (X1500).

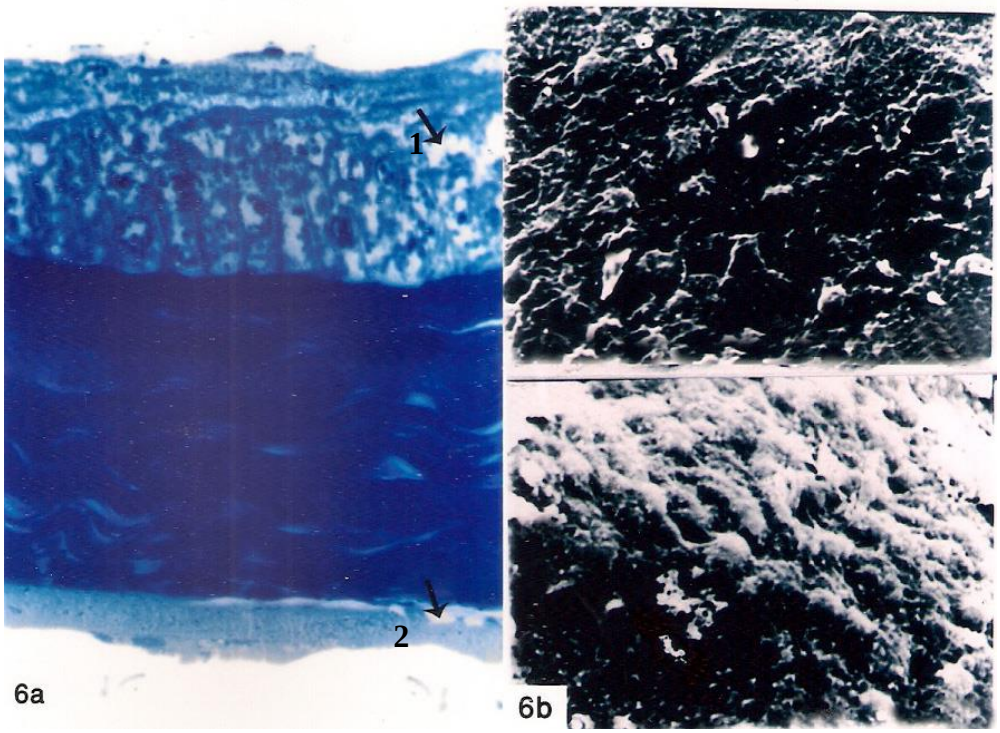

Figure (6): 72 hrs postmortem (a) light micrograph of cornea showed complete epithelial rarefaction with karyolysis (rupture of all cell membrane, nuclear membranes and only remenant of nuclear depris) (star) in addition to complete separation between superficial and deep epithelial layers (arrow 1). Also, most of endothelial cells were lost with areas of separation between Descemet's membrane and stroma (arrow 2) (X1250). (b) SEM examination showed that all the superficial cells were desquamated, some of the deep cells showed damage. Some of endothelial cells appeared swollen while others were altered with destroyed cell membrane.

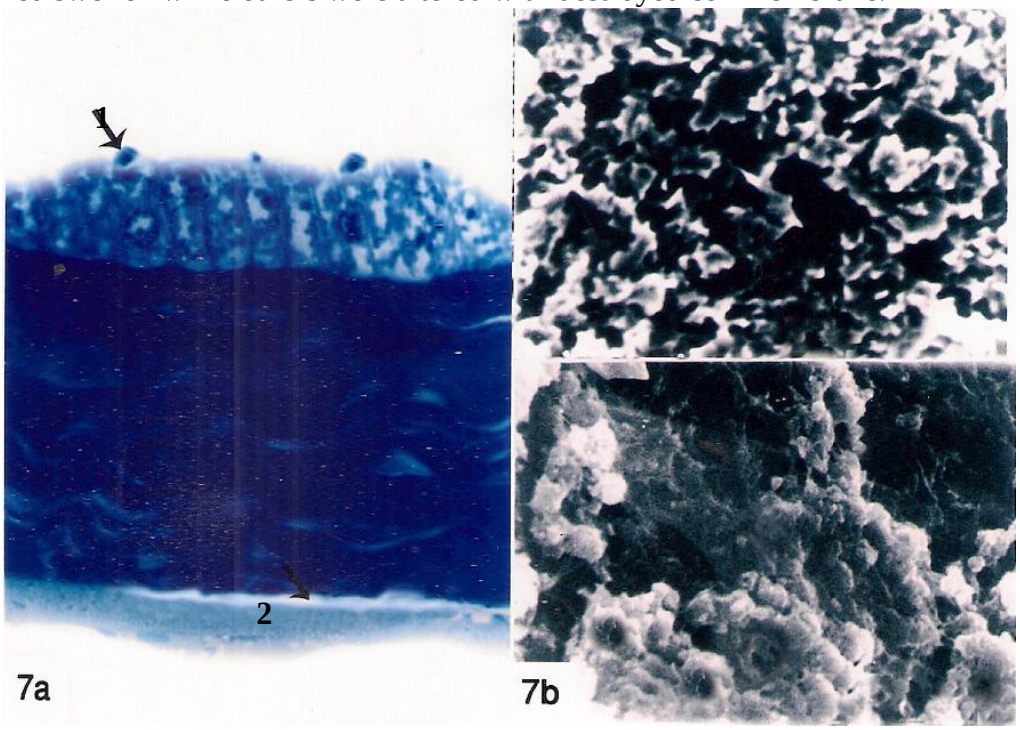

Figure (7): 96 hrs postmortem group (a) light micrograph of cornea: the epithelium showing only clusters of basal cells attached to cell membrane (arrow 1). Most of Descement's membrane is separated from stroma (arrow 2). No endothelial cells were observed (X1250). (b) SEM examination confirmed these results. The epithelium layer and most of endothelium showed loss of cyto-architecture pattern (X1500).

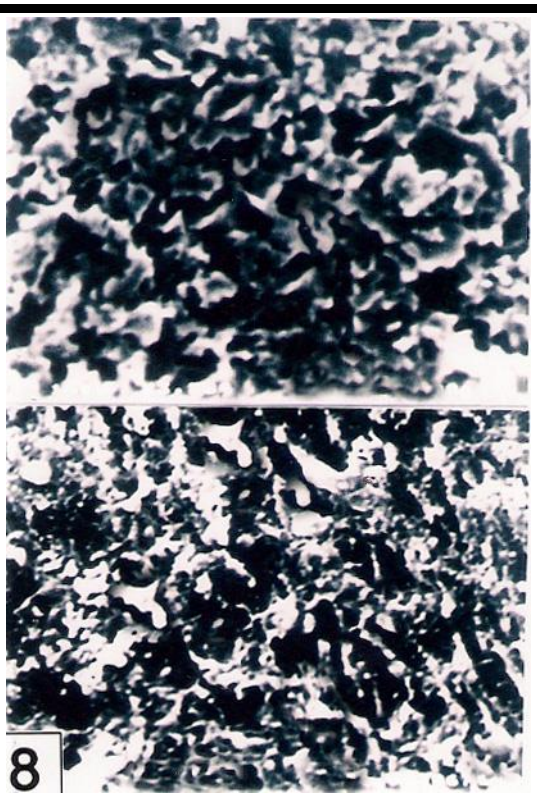

Figure (8):120 hrs postmortem group in which SEM examination confirmed the previous autolytic results as both epithelium and endothelium showed loss of the cytoarchitecture pattern (X1500).

DISCUSSION

The accurate prediction of time of death is of great value in medico-legal investigation of serious crimes, but the exact time of death cannot be fixed by any method because there are considerable biological variations in the individual cases [13]

The eye is considered as an important organ in estimation of the postmortem interval. In this study, rats were divided into two main groups each was subdivided into 6 subgroups according to known equal postmortem intervals (0, 24, 48, 72, 96 & 120 hrs). The 1st group was used for potassium analysis in vitreous humor and the 2nd used for detection of corneal changes that observed histopathologically by light and electron microscope at the same time intervals.

Vitreous humor is a biofluid of interest in several fields including ophthalmology, internal medicine and forensic pathology. The single most accurate biochemical method in estimation of time since death is the potassium contents in vitreous humor that indicates a strong correlation with the PMI [22&23].

In the present study, the 1st group showed a significant relation between potassium levels in vitreous humor (VH) and postmortem interval (PMI). The least levels were found at zero time then it increases gradually in a linear fashion to reach its highest levels at 120 hours postmortem.

During lifetime, potassium is almost intracellular. High intracellular concentration is maintained by Na+-K+ pump. After death this pump does not operate, therefore K+ is leaked out of cell, leading to high postmortem levels. Also, it is postulated that normal ante-mortem entry route of potassium into vitreous humor is through ciliary body. After death, autolysis of the vascular choroids and retinal cells are responsible for its rise [24].

Madea and Musshoff [25] explained this result after death as cell membrane become permeable, active and selective membrane transport stops, and the loss of selective membrane permeability with diffusion of ions, and other parameter according to their concentration gradient starts. In addition, **Mihailovic et al. [26]** stated a return to equilibrium at a steady rate after death due to the semi-permeability of cell membrane, so potassium immediately begins

to increase overtime for up to 120-125 hours after death.

Garg et al. [27] Concluded that potassium levels were found to increase up to 104 hrs. Other studies showed the same significant correlation, as the mean values of potassium concentration in VH of this study was consistent with that of **Teyin et al. [28]** who reported a significant relationship between potassium level and postmortem interval.

Jashnani et al. [9] reported that although vitreous fluid is stable for a longer periods postmortem, certain vitreous elements will change with diffusion from the retinal cells into the vitreous and the level of potassium increases linearly with increasing period after death until equilibrium with the plasma sets in [29].

In consistence with this study, **Collins [30]** stated that vitreous fluid could be procured up to approximately 4 days after death. Also, **Ahi and Garg** [31] observed that potassium level increase over time for up to 120-125 hours after death and this occur within the range of 21-40°C.

The variations in the slope of rise of K+ level in this study was different from McNeil et al [32] and this could be attributed to the differences in the ranges of postmortem interval observed in each study, in addition to vitreous humor storage procedures, number of cases and analytical techniques.

Lange et al. [33] analyzed data from six different studies on vitreous potassium and they stated that use of postmortem chemistry in the determination of PMI was difficult due to the generally small number of cases available to a single investigator and the effects of other factors. **Coe [8]** observed the external and internal factors that affect the postmortem vitreous biochemically. The external factors are sampling techniques; instrumentation and the environmental temperature of the body during the postmortem interval while the major internal factors are the age of the individual, manner of death and presence or absence of nitrogen retention.

Other studies were inconsistent with this study because they were pointed out that vitreous might be unsuitable for biochemical and toxicological analysis because of its abnormal viscosity and cellular composition or if the deceased had suffered from some diseases of the eye and had undergone ophthalmic operation. These obstacles were overcome in this study by exclusion of rats with any eye diseases or any chronic diseases, which might affect potassium level as renal failure [9].

Recent studies proved that the use of postmortem vitreous biochemically for the postmortem interval estimation has been limited because of the different conclusions reached by different workers and the lack of uniformity in their equations, so concluded the needs for farther researches to bring into use [34].

However, Chen et al. [35] were in agreement with this study, they concluded that vitreous humor is preferred because it has large volume, easily obtained and is usually free of contamination. It is relatively inert and slightly influenced by sudden fluctuations in the blood chemistry of the human body. So, this study supported a central role of vitreous humor biochemically in many postmortem forensic and pathological evaluations.

Regarding the importance of eye in forensic medicine in estimation of time since death especially its applicability in detection of donor corneas for transplantation, as it is harvested from cadavers and stored in eye banks, some researchers claim there is no upper time limit and donor corneas with extended postmortem times (i.e. intervals between death and preservation) can be used for grafting as long as certain quality criteria are met. The corneal endothelial cell density is considered to be the main factor in establishing the quality of corneal donor tissue [36&37].

Several reports have described the normal structure of corneal epithelium **Doughty** [**17&18]**, however very few studies had described the details of corneal epithelial and endothelial cells for approximate estimation of time since death.

In the present study, the 2nd group showed progressive degenerative changes in the cornea up to 96 hrs through histopathological picture either by light or electron microscope. Normal cornea with its five layers was found at zero time by light

microscope while, scanning electron microscopy at magnification X750 showed the polygonal superficial cells arranged in a mosaic pattern. However, 24 hrs postmortem showed slight degenerative changes in the corneal epithelium in the form of cloudy swelling (fluid accumulation inside the cytoplasm causing its vaculation).

The cells become flattened with loss of its palisading appearance while the endothelium was intact except few areas of rarefaction and these results were confirmed by scanning electron microscope. The pathogenesis of these cytoplasmic changes referred to mitochondrial damage with loss of ATP pump [38].

The cells of the corneal epithelium maintain several important functions including a barrier against abrasion and pathogens and this intact epithelium is essential for postoperative healing and graft transparency, the amount of donor epithelial cells may be correlated to the post-mortem harvesting time of the donor cornea. In most clinics, the donor epithelium remains on the transplant and this depend on postmortem intervals, which did not exceed 22 hours in most studies [39].

Cellular death is a process, which occurs gradually overtime depending on cells ability to survive anoxia caused by cessation of blood circulation and respiration. However, cornea is a special type of tissue, being avascular except the limbus irrigated by branches from anterior ciliary and the palpebral arteries [40].

The oxygen is supplied throughout diffusion by the precorneal tear film on the outer external membrane of the corneal epithelial cells [41]. Damaging effects of the tear film and disappearance of corneal epithelial cells integrity are caused by dehydration (especially when the eyes of the dead remain open), anoxia and acidosis from accumulation of lactic acid and pyruvate [42], all of which overlap and favor postmortem autolysis of the corneal cells, caused by proteolytic lysosomal enzymes [43].

These processes also cause a proportional alteration of the integrity of the tight junctions between adjacent epithelial cells, which normally seal the intercellular space preventing the diffusion of tear film components into the corneal deeper layers [44].

Regarding the applicability in forensic estimation of PMI, recent study used corneal florescence staining as a method for postmortem interval estimation [45]. This method is a cheap, fast, easy to use and non-invasive method, however it can detect postmortem interval less or more than 24hrs without determining the approximate time for estimation of time passed since death as this method depends on the percentage of the surface of stained area of the total corneal area. In the present study, degenerative changes in cornea can be detected by light microscope and correlated to time since death up to 96 hours as well as electron microscope that was correlated to time since death up to 120 hours.

Several studies have demonstrated that the corneal epithelium is well preserved for up to 4 weeks during organ culture storage [46].

Slettedal et al. [47] described the epithelial structure of donor cornea postmortem and showed for the first time that donor corneas with postmortem times of up to 7 days still have epithelial cells attached to the basement membrane, apparently without any structural damage to the cell membrane.

The difference in time since death from degenerative changes of the cornea especially the epithelium might be attributed to many factors like cause of death, nutritional status, general health condition, use of medication, diseases, previous eye surgery and contact lens wear, as well as cadaver treatment, storage temperature and eyelid position.

In this study, light microscopy of cornea demonstrated separation between the superficial cell layers and the middle layers (the superficial cell layers broke up and detached in the form of sheets) in specimens with post mortem times greater than 48 hours and this was in agreement with **Slettedal et al. [47].**

This finding proved that there is a weak adhesion between the superficial and intermediate layers of the epithelium and this was supported with **Crewe and Armitage [20]** who showed that tight junctional protein is limited to the superficial layer. Also, Bleb formation as a degenerative sign or as a result of apoptosis was prominent in the middle cell

layer and this separation was either due to this bleb formation or continuous exfoliation of superficial cell layers, which cause rapid disappearance of the middle layer cells.

In this study, only clusters of cells may be attached to the basement membrane at 96 hours postmortem, however, **Slettedal et al. [47]** showed that donor corneas had a rim of attached peripheral basal cells with postmortem times of 6-7 days. This may be due to cooling of tissues that may prevent the rapid breakdown of cells, as metabolism is then reduced.

The post-mortem disintegration of certain structural proteins or adhesion molecules may contribute to the loosening of cells. Another possible explanation is that the basal cells of the corneal epithelum are in fact weakly attached to the central cornea due to centripetal movement of cells, which is a very slow movement [49].

CONCLUSION

Estimation of time since death can be detected through chemical determination of potassium level up to 120 hours postmortem. Also histopathological changes in rat's cornea by light microscope can detect time since death up to 96 hours postmortem for corneal epithelium and 72 hours postmortem for corneal endothelium, while by scanning electron microscope (SEM) both corneal epithelium and endothelium could be detected up to 96 hours postmortem.

Further studies are necessary to determine the maximum limit for potassium level and other elements. Also, for determination of the exact time of epithelial and endothelial cell survival postmortem and the rate of epitheleal regeneration for detection of proper time for corneal transplantation

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علاقة كلاً من مستوى البوتاسيوم في السائل الزجاجي للعين والتغيرات الهستوباثولوجية لقرنية الجرذان البيضاء بالفتره ما بعد الوفاه

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المقدمة: مازال تقدير فترة ما بعد الوفاه من القضايا التي لها أهمية كبيرة في مجال الطب الشرعي وتعتبرالعين ذات دلالة طبية شرعية في هذا المجال. **الهدف من البحث:** علاقة كلاً من مستوى البوتاسيوم في السائل الزجاجي للعين والتغيرات الهستوباثولوجية للقرنية بالفترة ما بعد الوفاه في الجرذان البيضاء. **المواد المستخدمة وطريقة البحث:** تم استخدام 60 فأر قسمت بالتساوي إلى مجموعتين أساسيتين. المجموعة الأولى: قسمت إلى 6 مجموعات فرعية متساوية حيث تم سحب عينات السائل الزجاجي من العين لتحديد نسبة البوتاسيوم بها وأخذت العينات السليمة بعد استبعاد أي إصابة أو مرض بالعين. تم تحليل نسبة البوتاسيوم بعد 0، 24، 48، 72، 96، 120 ساعة . المجموعة الثانية: قسمت أيضاً إلى 6 مجموعات فرعية متساوية حيث تم استئصال قرنية الجرذان وذلك لتحديد التغيرات الهستوباثولوجية للقرنية باستخدام الميكرسكوب الضوئي والميكروسكوب الإلكتروني والمقسمة على نفس الفترات السابقة عند درجة حرارة 20 مئوية.

النتائج: أظهرت النتائج الكيميائية زيادة نسبة البوتاسيوم بشكل تدريجي مع كل فترة زمنية بعد الوفاة لتصل أقصاها بعد 120 ساعة. كذلك أظهرت الدراسة الهستوباثولوجية باستخدام الميكرسكوب الضوئي حدوث تغييرات في كل طبقات القرنية حيث تبدأ الطبقة السطحية في التآكل عند 24 ساعة بعد الوفاه ويزداد ذلك تدريجياً بحدوث انفصال جزئي بين طبقاتها إلى أن يحدث موت وفقد كامل لتلك الخلايا على هيئة صفوف ويتبقى مجرد تجمعات من الخلايا القاعدية مرتكزة على غشاء الخلية عند 96 ساعة بعد الوفاه بينما تتأثر البطانة الداخلية للقرنية تأثراً طفيفاً عند 24 ساعة بعد الوفاة وتستمر في التحلل بحدوث فراغات في السيتوبلازم إلى أن يتم موت وفقد كامل لتلك الطبقة عند 72 ساعة بعد الوفاه. وقد أكد الميكرسكوب الإلكتروني تلك التغيرات بالطبقة السطحية لقرنية العين عند 24 ساعة بينما كانت البطانة الداخلية سليمة في تلك الفترة ثم ازدادت التغيرات تدريجياً في كلا منهما نظراً لزيادة تحلل أنسجة القرنية بمرور الوقت ليفقدا التركيب الخلوي لكلاهماعند96 – 120 ساعة بعد الوفاه.

الخلاصة: من الممكن استخدام السائل الزجاجي للعين لتقدير فترة ما بعد الوفاه من خلال تحديد نسبة البوتاسيوم بهذا السائل والتي تزداد تدريجياً بزيادة فترة ما بعد الوفاه إلى حد يصل إلى 120 ساعة. كما أثبتت التغيرات الهستوباثولوجية التي تحدث لقرنية العين بعد الوفاة باستخدام الميكروسكوب الضوئي والإلكتروني إمكانية تقدير فترة ما بعد الوفاه بحدود 96 ساعة لذلك من الممكن الإعتماد على إحدى الطريقتين أو كلاهما في تقدير فترة ما بعد الوفاه مع الأخذ في الإعتبار أهمية التغيرات الهستولوجية في حالات زرع قرنية العين.