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Variation of the intraocular pressure in relation to time of death: contribution to the determination of the true postmortem interval (PMI)

Original Article

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SUMMARY

Introduction: *Postmortem Interval estiamtion is a measure of singular importance in Legal Medicine. Due to its complexity, it needs objective evaluation in Forensic Pathology.*
Methods: *In this study we propose the use of a bovine model to sytematically measure premortem and postmortem Intra-Ocular Pressure (IOP) as well as analyze its evolution with time as a possible non-invasive method to estimate PMI. A prospective, descriptive, observational study was carried out, using 12 cows. For the determination of the IOP, the aplanatic manual Perkins tonometer calibrated for bovines was used. The measurement of the IOP was done at establisehd post-mortem intervals.*
Results: *The mean IOP at hour 0 in OD was 13.48 mm Hg and 13.59 mm Hg in OS with minimal variation between groups; at hour 30 hr, the IOP was 6.41 and 6.45 mm Hg in OD and OS respectively. We observed a decrease of the IOP in al leyes, directly related to the elapsed time of death.*
Discussion. *The uniformity of the IOP estimations in relation to postmortem interval allows us to consider it as a valuable parameter for postmortem interval even several hours after death.*

Keywords*: Postmortem Interval, IntraOcular Pressure, tonometry.*

INTRODUCTION

Determination of postmortem interval (PMI) is a data of singular importance in Legal Medicine, constituting by its complexity one of the problems that needs objective evaluation in Forensic Pathology. Calculation of PMI must be based on measures that are capable of reproduction, revision and criticism. Hence the importance of reducing the subjective and personal nature of the assessments and their replacement by instrumental records based on scientific evidence[1].

The accuracy and applicability of procedures to study physicochemical, biological and microbiological phenomena depend on the characteristics and circumstances of death and post-mortem interval. The decrease in temperature is usually mentioned more frequently; the rigor mortis; the cadaverous lividity; the acidity of fluids such as blood, urine and cerebrospinal fluid; the autolysis and finally the putrefaction. The joint use of biochemical methods and the determination of the temperature improve the estimations whenever they are carried out in the first 24 hours of the death; accuracy decreases proportionally to the elapsed interval[2].

It is then intended to extract samples from closed places with little possibility of post-mortem contamination, that is, outside the general circulation, with the eyeball being a valid option[3].

The general method to determine the postmortem interval in a forensic scenario is the categorization of the amount of decomposition within the different stages, where the eye is the least degraded in the usual samples[4]. According

to Luna et al, it could be affirmed that in the first 24 hours postmortem, the combination of the study of potassium in the ocular vitreous humor and the rectal temperature offers acceptable results, which could be improved with the incorporation of other complementary elements[5]. The vitreous humor is an inert, transparent material that forms a gelatinous structure in the four fifths of the posterior part of the eyeball. It contributes to the optical functions during life and helps in the evaluation of post-mortem interval after death. Jaffe (1962) observed for the first time the increase of potassium in the vitreous during IPM on a regular basis and the average rate of increase was 0.17mEq / hr[6]. Blumenfield (1974) reported a linear increase in potassium concentration in relation to IPM[7] while Govekar (1997) reported that there is a linear increase in potassium values ranging from 3.56 mEq / L to 15.5 mEq / L[8]. However, other authors have questioned this methodology based on differences between both eyes and significant variations in the different measures [9-10]; therefore, other measurable ocular parameters should be introduced in these situations.

Intraocular pressure (IOP) is the result of a balance between production and elimination of aqueous humor. Regardless of the manner and the intimate mechanism of formation of aqueous humor, several authors have determined that in humans the flow of aqueous humor is around 2 µl / minute[11]. This mechanism, which is supposed to be suspended at the time of death, could gradually diminish the production of aqueous humor and, therefore, intraocular pressure. Our determinations of the IOP obtained in previous work, in about 70 bovines indicated a surprising constancy of 16 mm Hg[12].

Based on this fact, it is proposed in this study to use this bovine model for the taking of pre and post-mortem intraocular pressure and to analyze its evolution over time as a possible non-invasive approach to IPM.

METHODS

A study was carried out in a single center, of a prospective, observational, descriptive type, with animals destined for slaughter in the Municipal Slaughterhouse of the City of Corrientes, Argentina; posterior determinations were performed in the Faculty of Medicine of the National University of the Northeast (UNNE), in the Argentine Republic. We used 12 cows, from 3 to 5 years of age, with a weight of 350 to 420 kg, of Bradford breed (crosses between Brahman and Hereford). Each animal was identified with a number in the ear lobe. For intraocular pressure measurement, the following protocol was used: topical anesthetic instillation (Proparacaine 0.5%, Anestalcon®) and a dye (Fluorescein 0.25%); as a measuring instrument, the manual aplanatic tonometer (Perkins) used in humans was used, previously calibrated for the measurement of IOP in the bovine eye as detailed below:

Precalibration of the Perkins Tonometer for IOP measurements in cows eyes

Four cow eyes were obtained from the local municipal slaughterhouse. The eyes were transported on ice immediately after being enucleated. It was verified that all the eyes were intact with their transparent corneas. The eyes were cannulated with a number 26 needle perpendicular to the visual axis 1-2 mm

anterior to the sclero-corneal limbus. A low magnification microscope was used to perform the cannulation. It was verified with the microscope that there were no fluid losses. The IOP was controlled by adjusting the height of the fluid column in the pipette connected to the needle inserted in the eye. The IOP was continuously monitored and recorded with a pressure translator (Ohmeda model TNF-R) connected to a second needle inserted in the anterior chamber. The IOP was adjusted to 15, 25, 35, 45 and 55 mm of water by changing the height of the pipette with its fluid column. The eyes were placed in a small container that allowed the anterior part to protrude. In this position the IOP was taken, noting the value indicated by the Perkins tonometer. Five measurements were made at each pressure level. The measurements were plotted to convert the numerical value of Perkins with the real IOP of the eye as shown in figure 1 previously published by this research group[12].

Figure 1 shows the comparison between measurements of the Perkins tonometer and direct intraocular cannulation. The measurements of the Perkins Aplanatic tonometer is a number on the scale of the instrument designed for measurement in human eyes. Each point of each level of IOP is the average of 5 measurements on 1 to 4 bovine eyes. The Pearson correlation coefficient resulted in 0.96.

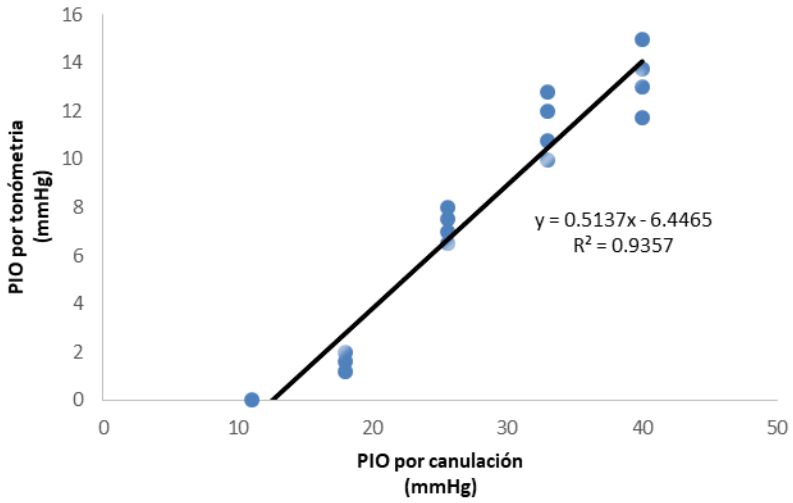

Figure 1. Comparison of IOP measurement results with the Perkins tonometer and direct intraocular cannulation. N = 12

Once the tonometer was calibrated, IOP was taken in the selected animals that were guided by a brete that had a final device that closes around the neck (clamp) to immobilize the head and allow the first measurement to be made. This was considered the base measurement (hour 0) during the animal's life, strictly determining the time of slaughter for each case.

We proceeded to the section of the heads of the animals under study, which were transferred to the UNNE School of Medicine to continue with IOP measurements, which were stored in the

laboratory at room temperature. IOP measurements were repeated at the time, and at 3, 4, 8, 12, 16, 20, 24 and 36 hours from the time of sacrifice. The European Community Norms on animal research were respected, also with the approval of the responsible veterinarian.

Data Analysis

The importance of the changes observed in the IOP measurements and the evolution of time were analyzed using the Student's t test, with <0.05 chosen as the level of significance.

RESULTS

The results of the IOP measurements as a function of time were ten measurements per eye and the data are presented in table 1 for the right eye (RE) and in table 2 for the left eye (OS).

Hours	*0*	*1*	*3*	*4*	*8*	*12*	*16*	*20*	*24*	*36*
#108	13,73	13,73	10,72	10,29	9,00	8,14	8,14	7,71	7,28	6,41
#109	13,73	12,87	10,29	9,43	9,43	9,00	8,57	8,57	8,14	6,84
#110	13,73	13,73	9,43	9,43	9,00	8,57	7,71	7,28	7,28	6,41
#111	12,44	11,58	9,43	9,43	9,00	9,00	8,14	7,28	7,28	6,41
#112	13,73	13,73	12,01	12,01	10,29	9,43	8,57	7,28	7,28	6,41
#113	13,73	12,87	12,44	11,58	11,58	10,29	9,43	7,71	7,71	6,84
#114	12,87	12,01	11,58	10,72	9,43	8,57	8,57	8,14	7,28	6,41
#115	13,73	13,73	12,44	12,01	11,15	9,43	8,57	8,14	7,28	5,98
#116	12,87	12,87	12,01	11,58	10,72	9,43	8,57	8,14	7,28	5,98
#117	14,6	13,73	13,73	12,87	12,01	10,29	9,43	8,14	7,28	6,41
#118	13,73	13,73	12,87	12,44	11,15	9,43	8,57	7,71	7,28	6,84
#119	12,87	12,44	12,01	11,58	10,29	9,43	8,57	7,71	6,84	5,98
Aver	13,48	13,09	11,58	11,11	10,25	9,25	8,57	7,82	7,35	6,41
SD	0,59	0,77	1,35	1,22	1,08	0,65	0,49	0,42	0,31	0,32
ES	0,178	0,230	0,403	0,366	0,322	0,194	0,145	0,124	0,093	0,095

Table 1. Intraocular Pressure of the Right Eye. Own source.

Hours	*0*	*1*	*3*	*4*	*8*	*12*	*16*	*20*	*24*	*36*
#108	13,73	12,87	11,58	10,72	9,43	8,57	8,14	7,71	7,28	6,41
#109	13,73	13,3	11,58	10,29	9,43	9,00	8,14	8,14	7,28	6,84
#110	13,73	13,73	11,58	10,72	9,00	8,57	7,71	7,71	7,28	6,41
#111	13,73	12,87	12,01	11,15	9,43	9,00	8,14	7,71	7,28	6,41
#112	12,87	12,44	12,01	11,15	10,29	9,43	8,57	7,71	7,28	6,41
#113	13,73	12,87	12,44	11,58	11,15	9,43	8,57	7,71	7,71	6,84
#114	13,73	12,87	12,44	11,58	10,29	9,43	8,57	8,14	7,28	6,41
#115	13,73	12,87	12,44	12,01	11,15	10,29	9,43	7,71	6,84	5,98
#116	13,3	12,87	12,44	12,01	11,15	9,43	8,57	8,14	7,28	5,98
#117	13,73	12,87	12,01	12,01	10,72	10,29	9,43	8,14	7,28	6,41
#118	14,16	13,73	13,3	12,87	11,58	10,72	9,43	8,57	7,71	6,84
#119	12,87	12,44	12,01	11,58	10,72	9,86	9,00	7,71	7,28	6,41
Aver	13,59	12,98	12,15	11,47	10,36	9,50	8,64	7,93	7,32	6,45
SD	0,38	0,42	0,50	0,71	0,86	0,68	0,57	0,29	0,22	0,29
ES.	0,114	0,124	0,149	0,213	0,257	0,204	0,172	0,087	0,067	0,086

Table 2. Intraocular Pressure of the Left Eye. Own source.

The mean IOP at hour 0 in OD was 13.48 mm Hg and 13.59 mm Hg in OS with minimal variation between the different cases. The slope is uniform and similar in both eyes. The average final value at 36 hours was 6.41 and 6.45 mm Hg in OD and OS respectively, with little variation between the eyes of the 12 animals studied.

Both eyes had a similar behavior regarding the decrease in IOP as the hours passed. The averages of all the DO and the OS show a decrease of similar characteristics that can be seen in figure 2. In the case of the DO, the IOP decrease is of the order of 0.79 mmHg for each change in the studied interval and in the LE, it was -0.81 for each change in the interval studied.

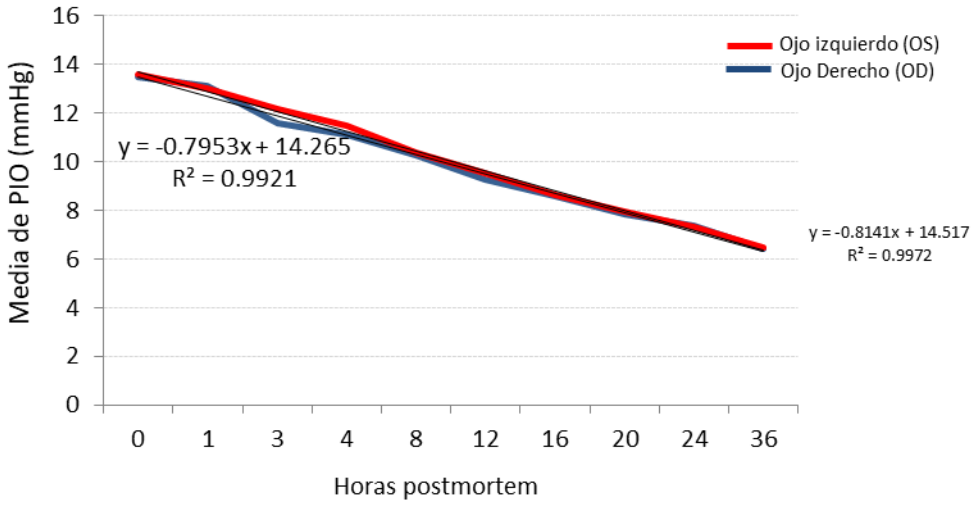

Figure 2. Mean IOP change per postmortem hour, according to the eyes of animals analyzed (n = 12).

DISCUSION

A decrease in IOP is observed as the time of death elapses, in a regular manner, in all eyes. The measurement is effective until the eye lowers its pressure and loses its consistency, which usually occurs 60 hours after sacrifice.

The fact of the uniformity of the decrease in IOP as a function of time would allow an IOP taken hours after death to be extrapolated at the time of death. This procedure could be associated with other tests already established to access the postmortem interval with greater consistency.

Eventually this protocol could be used in corpses to collaborate in determining the moment of death or, in the words of Verdú, of the true death interval (VIM) [13].

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