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Research Article

BIOCHEMICAL ANALYSIS OF AQUEOUS AND VITREOUS HUMOUR OF JAPANESE QUAILS *(COUTRNIX JAPONICA)*

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ABSTRACT
A study on biochemical characterization of aqueous humour and vitreous humour of Japanese quail (*Coturnix japonica*) was carried out. A total of 12 eye ball samples were collected from Japanese quail from a slaughter house near kumbakonam. The aqueous and vitreous humours were separated by adopting aseptic procedures and were collected in clean vials. Biochemical analysis of aqueous and vitreous humour revealed that glucose, total proteins, albumin, creatinine, calcium and enzymes like ALT, AST, ALP were greater in vitreous humour than in the aqueous humour. Phosphorus content was lesser in aqueous humour then in vitreous humour. Sodium and urea levels are almost same in both aqueous and vitreous humours. The Urea nitrogen content in aqueous and vitreous humour was 7 mg/dL and 8.4 mg/dL in vitreous humour. Hence the vitreous humour could be used as a medium for analysis of post-mortem Urea concentration.

Keywords: *Cysticercus Tenuicollis, Cystic Fluid, Biochemical Analysis, Goats*

INTRODUCTION
Eyes are generally adapted to the environment and life requirements of the organism which bears them. Eyes are the organs of vision. They detect light and convert it into electro-chemical impulses in neurons. The eye is architecturally and biochemically organized to maintain high-quality vision. In order to function as organ of vision it is of vital importance for the eye to maintain a highly regulated environment for the visual cells and transparent tissues. Consequently, tight cellular barriers, which restrict and regulate the uptake of fluids and solutes, are present in the anterior and posterior parts of the eye. Aqueous humor provides a transparent and colorless medium between the cornea and the lens and constitutes an important component of the eye's optical system. The aqueous humor is a transparent, gelatinous fluid similar to plasma, but containing low protein concentrations.
Vitreous humour (VH) is a transparent, highly hydrated gel, which occupies the posterior segment of the eye between the lens and the retina. It is comprised almost entirely of water (99%) with the remainder consisting of a mixture of collagen fibres, hyaluronic acid, hyalocytes, inorganic salts, and lipids. The vitreous is the transparent, colourless, gelatinous mass that fills the space between the lens of the eye and the retina lining the back of the eye. It is present at birth and does not change much over the course of aging. Amazingly, with so little solid matter, it tautly holds the eye. The lens, on the other hand, is tightly packed with cells. However, the vitreous has a viscosity two to four times that of pure water, giving it a gelatinous consistency. It also has a refractive index of 1.336. Unlike the fluid in the frontal parts of the eye (aqueous humour) which is continuously replenished, the gel in the vitreous chamber is stagnant.
There is evidence suggesting that postmortem eye fluid analysis could provide a reliable estimate of ante mortem chemistry values. Eye fluid, which is a filtrate of the blood, is in a protected anatomical location and is infrequently contaminated by blood during collection. In human medicine, postmortem eye fluid has been used to estimate the time of death (postmortem interval), antemortem serum chemistry values, antemortem drug and toxin levels, and to investigate "sudden infant death syndrome.
The importance of post-mortem eye fluid analysis has not been realized, at least in the sudden deaths after several intoxications. As a prelude to the beginning of a new era involving the analysis of the eye ball

fluids in post-mortem, the present study was planned to carry out the estimation of biochemical analytes in the aqueous and vitreous humour of Japanese quails.

MATERIALS AND METHODS

A total of 12 eye ball samples were collected from Japanese quail (*Coturnix japonica*) from a slaughter house near kumbakonam. The samples were collected immediately after slaughter, and brought to the laboratory in an ice box following complete aseptic measures. In the laboratory, the aqueous humour was first removed with a sterile syringe and collected in a sterile container. A transverse cut was made in the cornea and the lens with the capsule expelled and collected in a sterile bottle. The vitreous was then pressed out through the cornea and also collected in a sterile bottle. The time taken from the collection of the eyes at the slaughter house to processing in the laboratory was not more than 2 hours.

Aqueous Humor: A 26 gauge needle was fixed to a 5 mL syringe and it was inserted into the anterior chamber through the corneal limbs. Aqueous humor was aspirated and collected from each eye. A protease inhibitor cocktail (sigma) was added immediately to prevent degradation of proteins. The samples were placed on ice and centrifuged at 5000 rpm for 10 minutes.

Vitreous humor: The vitreous humor was collected by grasping the vitreous with forceps after opening of the eyeball by a lateral incision at the corneal limbus. The vitreous was centrifuged for 10 minutes at 5000 rpm, and the vitreous humor (the supernatant) was collected and stored at -20^{o}C.

Biochemical assays were carried out for Glucose, total Protein, Albumin, Urea, Creatinine, Total Cholesterol, Triglycerides, ALT, AST, ALP, Calcium and Phosporous. These parameters were investigated with Span Diagnostic kits as per the standard biochemical procedures (Kaneko *et al.,* 1997).

The total protein content of the samples was estimated the by Biuret method (Gornall *et al.*, 1949) with slight modification. A standard curve was built using Bovine serum Albumin (BSA) as standard (10mg/mL).

RESULTS AND DISCUSSION

The aqueous humour and the vitreous humour collected from the eye balls of Japanese quails were subjected to several biochemical estimations and the results are tabulated as in Table 1.

Table 1: Biochemical composition of aqueous and vitreous humour

Parameters	Aqueous Humor	Vitreous Humor
Glucose (mg/dL)	60±1.08	80±2.16
TP (g/dL)	0.76±0.06	0.89±0.12
Albumin (g/dL)	0.24±0.09	0.44±0.14
Urea (mg/dL)	15±1.14	18±1.26
Creatinine (mg/dL)	0.86±0.04	1.08±0.02
Total cholesterol (mg/dL)	20±2.44	34±3.67
Ca (mg/dL)	5.8±0.08	6.4±0.14
P (mg/dL)	3.1±0.12	2.8±0.26
ALP(U/dL)	12.95±1.14	18.69±3.14
AST(U/dL)	44±5.64	60±3.34
ALT(U/dL)	28±4.18	54±5.17
Na(mM/dL)	152±7.08	148±6.04

The result revealed that the levels of biochemical constituents in aqueous and vitreous humour were not uniform. There was difference between the levels of biochemical constituents between these fluids and serum. Henke and Demarais, 1992 reported that the vitreous humour concentrations of glucose, triglycerides, sodium, potassium, cholesterol, total protein, albumin, lactate dehydrogenase, creatine kinase, aspartate transaminase, bilirubin, cortisol, and IgG were neither similar to nor predictive of serum constituents. Glucose, Total protein, Albumin, Creatinine, Calcium and enzymes like ALP, ALT, AST

were greater in the vitreous humour than the aqueous humour. Kachhawaha *et al.,* 2004 reported 15.03 mg/dL glucose and 8.55 mg/dL of calcium in the vitreous humour in buffalo Calves and also reported that the value of glucose decreased five folds after six hours of slaughter. Yahia and El-Hakiem, 2014 reported that the level of glucose in vitreous humour of donkeys as 55.85mg/dL. Fakhruddin *et al.,* 2003 reported almost similar levels of glucose in aqueous humour and vitreous humour at 0 hour slaughter in goats. Protein content was lesser in aqueous humour than vitreous humour. Jashnani *et al.,* 2010 reported that age, sex, cause of death, season of death, and refrigeration of sample did not influence the vitreous humour potassium values. Mulla *et al.,* 2005 demonstrated that the between-eye differences for vitreous electrolytes and calcium are insignificant. The results of the lipid contents of aqueous humour was lesser than vitreous humour, are in accordance with the earlier reports of Zygulska-Mach *et al.,* 1993 in rabbits. Chen *et al.,* 2009 observed that levels of chemical components in human vitreous humour are changed with time after death, which can help to estimate the PMI. But in the present study, the Sodium and urea levels are almost same in both the aqueous humour and vitreous humour. Hanna *et al.,* 1990 concluded that an accurate estimate of antemortem serum urea or creatinine can be made from the analysis of aqueous or vitreous fluid at necropsy. The Urea nitrogen content in aqueous and vitreous humour was 7mg/dL and 8.4mg/dL in vitreous humour. Raja *et al.,* 2011 reported the urea level in jersey cross red animal and as 37.73mg/dL in vitreous humour of cattle. The lower level of urea content is related to the species of study, as earlier reports of Henke and Demarais, 2001 studied in male black-tailed jackrabbits (*Lepus californicus)* suggested that the vitreous humour constituents were similar or linearly correlated to serum constituents for urea nitrogen, triglycerides and glutamic-oxaloacetic transaminase. The remaining vitreous humour constituents were neither similar nor predictive of serum of constituents. Schoning and Strafuss, 1980 observed in 60 adult mongrel dogs that Sodium, chloride, and urea nitrogen values were stable at 4 degrees C for 48 h. Hence the vitreous humour Nitrogen contained could be used as medium for analysis of post-mortem Urea concentration and to assess the time interval after death.

A comparative study of ante-mortem and post-mortem values of biochemical analytes may be carried out to confirm our results. Serum samples may be collected simultaneously from same animal to understand the variations in the distribution of different biochemical analytes.

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