

CHARACTERIZATION OF AQUEOUS AND VITREOUS HUMOUR PROTEINS OF JAPANESE QUAIL (*COTURNIX JAPONICA*) THROUGH SDS-PAGE

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ABSTRACT

The nature and patterns of proteins present in the aqueous and vitreous humor proteins of Japanese quail (*Coturnix japonica*) was studied in samples collected from the slaughterhouse eyeball specimens through Sodium dodecyl sulphate - Polyacrylamide gel electrophoresis. The discontinuous SDS-PAGE (10% gel) revealed at least five prominent bands in aqueous humor and eight prominent bands in vitreous humor of Japanese quails. At least three fainter bands in aqueous humor and five fainter bands in vitreous humor were also observed. In aqueous humor, all the prominent bands were within the range between 66kDa to 10kDa (66KDa, 45kDa, 20kDa, 15kDa and 10kDa). The fainter bands were observed at 220kDa, 50kDa and 30kDa. Among all the bands which were observed as triplets at 10-20kDa were very prominent followed by the 66kDa band. The resolution of the band was sharper, discrete and non-overlapping. In vitreous humor eight prominent bands were observed at 220KDa, 66KDa, 50KDa, 45kDa, 30kDa, 20kDa, 15kDa and 10kDa. Five fainter bands were observed at 135kDa, 120kDa, 80kDa, 25kDa and 5kDa. Among all these bands the triplet bands which were found in the lower part of the gel were very prominent (20kDa, 15kDa and 10kDa). Similar to the aqueous humor but the level of proteins was higher than that of aqueous humor hence the triplet bands were found over lapping appearing as a very large single band. Between the 20kDa and 66kDa, 3 prominent bands of 50kDa, 40kDa, 30kDa were observed. Among these 3 bands, the 50KDa and 40kDa bands were found to be clear discrete bands. The 66kDa and 220kDa were resolved sharply and were found to be single bands. Among the fainter bands, three bands were of greater than 66kDa size and two were of small molecular weight lesser than 25kDa. The pattern and number of bands were very different in aqueous humor and vitreous humor.

Keywords: Aqueous Humour, SDS-PAGE, Vitreous Humour, Japanese Quails

INTRODUCTION

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. These barriers are fundamentally important for the protection of the eye and for the maintenance of vital ocular functions. The aqueous humor is a transparent, gelatinous fluid similar to plasma, but containing low protein concentrations. 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. Proteins may accumulate in the vitreous by local secretion (e.g., glycoprotein), filtration from blood (e.g., albumin), or diffusion from the surrounding tissues. Unlike the fluid in the frontal parts of the eye (aqueous humour) which is continuously replenished, the gel in the vitreous chamber is stagnant. Therefore, if blood, cells or other by products of inflammation get into the vitreous, they will remain there unless removed surgically. A study was carried out to analyze the protein patterns of eye balls fluids through SDS-PAGE of aqueous and vitreous humor of Japanese quails.

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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. The samples were centrifuged at 3000 rpm for 15min. and the supernatants were separated and kept at -20°C until further use. The protein content of the samples was estimated by biuret method with slight modification. A standard curve was built using Bovine Serum Albumin (BSA) as standard. The photometric estimation was carried out with the help of ELICO SL 207 mini spectrophotometer. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by following the method of Laemmli (1970) with some modifications.

RESULTS AND DISCUSSION

The electrophoretogram obtained through discontinuous SDS- PAGE (10 % gel) was shown in the figure.

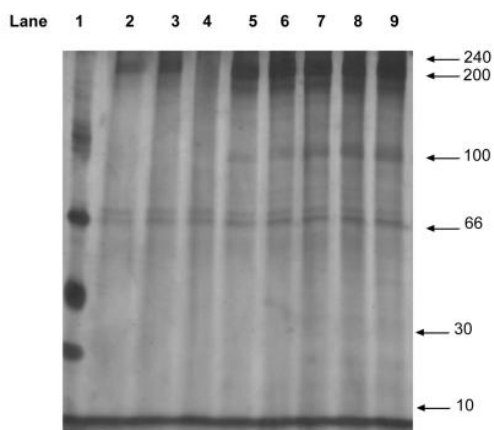


Figure 1: SDS-PAGE analysis of Vitreous Humor
Lane 1- Protine markers (Low molecular weight)
Lane 2-5 Aqueous Humor
Lane 6-9 Vitreous Humor

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Similar results were reported by (Zaidi *et al.*, 2010) as presence of (5 bands of 6kDa, 10kDa, 30kDa, 79kDa, and 90kDa size in the aqueous humor of human beings. Most of bands which were observed at the lower one there of the gel, were already reported by Kuchle *et al.*, 1994 as trains of protein bands at the range of 28kDa-48kDa in the calf aqueous humor. Chowdhury *et al.*, 2010 reported 8 prominent bands in the range of 15 to 250kDa and observed 3 to 4 doublets of protein bands below 66kDa size in human aqueous humor. Our results are in full agreement with the earlier reports of aqueous humor reports in human samples. The 66kDa band was found in aqueous humor albumin, which appeared as a transudate into fluid component.

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