

Trematode infection & granuloma formation – How basic research helps in confirming the clinical finding?

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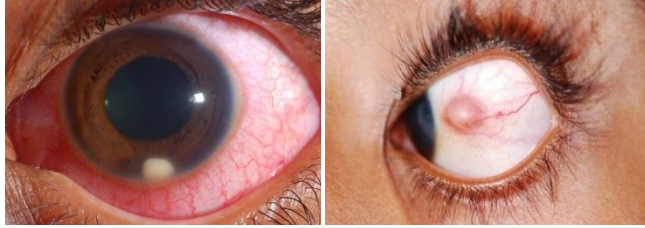
Ocular inflammation or Uveitis in children is a potentially vision threatening condition, the causes vary widely depending upon geographic, environmental and socioeconomic factors and also upon the prevalence of causative agents. Nearly 120 children come to our hospital with uveitis every year. Of 120, nearly 20 % come with granuloma either in subconjunctival space or in anterior chamber. All children who developed such granuloma give history of eye problem after bathing in river or ponds near the river Kavery which brings major water supply to east central part of Tamil Nadu state, suggesting a water-borne infection. Clustering of cases seen in several south Indian villages, over decades suggested a widespread distribution of the etiologic agent. We worked up all these patients clinically to find out the cause. The granulomas were surgically removed and subjected for histopathology, Presence of cuticle of a parasite on histopathology suggested a parasitic disease, possibly trematodes.

Parasite is a living organism that receives some of its basic nutritional requirements through its intimate contact with another living organism. When the parasite reaches the eye, ocular complications occur due to mechanical, immunologic or allergic reactions. When an ophthalmologist detects a parasite in the eye, often it is difficult to identify the exact organism. Confirmation of etiology and species identification is not possible by histopathological studies. Hence often the diagnosis is left labeled as parasitic disease, without any speciation.

However modern technological breakthrough has given us the tools which are more specific and persuasive than any other laboratory techniques. Using molecular technique such as Real-Time PCR - Syber green Assay, bi-directional sequencing and BLAST analysis identified the cause of the granulomatous uveitis as *Procerovum species* (Family- Heterophyidae) of trematode. Subsequently our

team visited the villages where these patients live and the snails were collected from water bodies. Trematode larvae were harvested from the live snails in our laboratory. RT PCR assay, molecular sequencing and BLAST analysis revealed the presence of *Procerovum* in the snails which is the intermediate host for these trematodes. This research work further confirmed the environmental source.

Exact etiology of this regional ophthalmic problem which was affecting children for more than 3 decades has been recognized by the basic research. The present work indicates the critical need for an epidemiologic investigation to learn the life cycle of the parasite, risk factors associated with the disease and finally the preventive strategies.



Anterior chamber granulomas

Subconjunctival granulomas

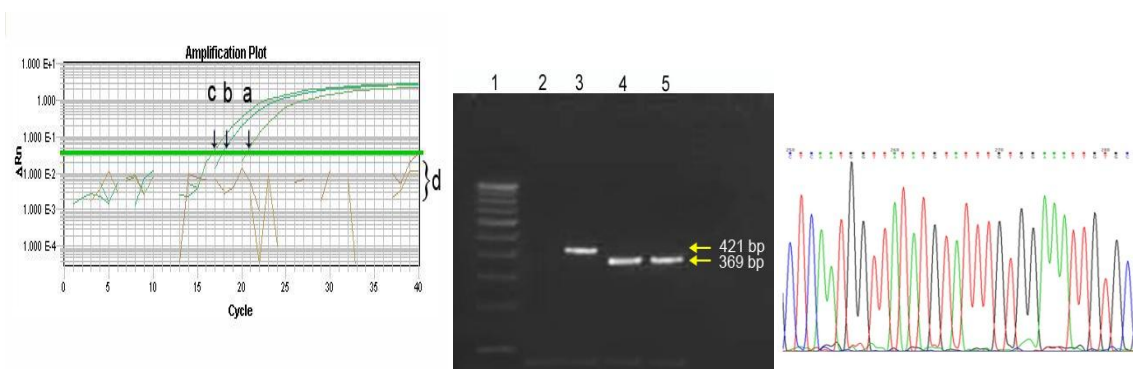


Fig 1(A). Real Time PCR-amplification of rDNA ITS2 regions of trematode
 (a) Positive control (*F.gigantica* DNA 100pg)
 (b) trematode cercaria DNA source Snails
 (c) Granulomas DNA from patients sample, and
 (d) Negative control (nuclease-free water)

Fig 1(B) . Gel electrophoresis of the amplified DNA checked on 2% agarose gel.
 Lane 1- 100bp DNA ladder- marker,
 Lane 2- negative control,
 Lane 3- positive control,
 Lane 4- Cercaria DNA, and
 Lane 5- Granulomas DNA of patients

Fig 1(C). Representative electropherogram that confirms the sequence of the trematode *Procerovum varium*



Fig 2(a). *Melanoides tuberculata* snails collected from the pond in the suspected focus of infection

Fig 2(b). Light microscopic images of cercaria larval stage recovered from the snails. X 200.