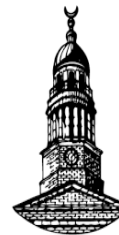




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CAIRO UNIVERSITY - FACULTY OF MEDICINE

Faculty of Medicine, Cairo University Postgraduate Research Protocol Template

(Please read carefully provided guidance documents for a comprehensive understanding and proper formulation of your thesis protocol and required forms)

1. Study

- a- Proposed Study Title: Ocular Fluid Work-up in Presumed Trematode Induced Granulomatous Uveitis
- b- Degree: MD
- c- Date of Registration of MSc or MD: June 2026

2. Candidate

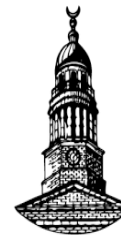
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4. Scientific committee approval

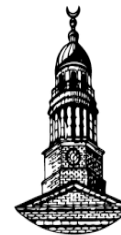
Date of approval: August 2026

5. Background and Rationale:

Presumed trematode-induced granulomatous uveitis (PTIGU) is a form of granulomatous uveitis affecting children in rural areas with access to contaminated, brackish, fresh waters particularly in developing countries such as Egypt and India. (1-2) The disease is characterized by granuloma formation anywhere in the eye, from the conjunctiva, anterior chamber (AC), and iris anteriorly to the ciliary body, choroid and retina posteriorly, and the more posterior the granuloma location, the worse is the prognosis. (3)

The characteristic presentation of anterior uveitis is with single or multiple pearly white AC nodules in one or both eyes that eventually evolve into vascularized retro-corneal membranes, anterior and posterior synechiae, cataract, and glaucoma. (4) PTIGU is also characterized by the development of the granuloma within the ciliary body (CB) and is divided into two stages: active stage with vitritis which if left untreated progresses to the cicatricial stage where vitreoretinal traction results in tractional retinal detachment (TRD). (5) Accordingly, diagnosis of such patients during the active stage is essential for early proper management to avoid complications that might eventually result in blindness. (9,10)

The term “presumed” is usually used to describe such uveitic entity since

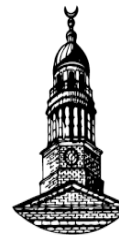


fragments and genetic material of the parasites could be isolated from only some of the surgically removed granulomas without signs of systemic parasitic disease (4,6,7,8) Children in South India who had a clinical picture of PTIGU had subconjunctival and AC granuloma analysis, which revealed DNA fragments of a digenetic trematode. Further sequencing identified *Procerovum varium*, as the causative organism species. (8)

In Egypt, molecular evidence of a digenetic trematode was found in 42.8% of the samples obtained from analysis of the excised intracameral granulomas. This was accomplished by conventional polymerase chain reaction (PCR) targeting mitochondrial cytochrome c oxidase subunit 1 (COI); a barcode for parasitic Platyhelminthes. (4) However, no further sequence analysis was done. Conventional PCR using the primer designed to target 28S rDNA, which was intended to detect *Procerovum varium*, was tested in Egyptian children with AC granuloma and yielded a negative result. (9) The digenic trematode *Schistosoma* is endemic in Egypt. Pathological examination of conjunctival granulomas in pediatric Egyptian patients revealed the presence of *bilharzia* ova. (11)

While digenetic trematode is suspected to be the cause of this type of induced uveitis, yet species may vary across different countries. In Egypt, further analysis by sequencing was not conducted to confirm the trematodal infection and identify the specific species responsible. In addition, no previous analysis of vitreous biopsies was done in any study.

There are several approaches to the management of presumed trematode-induced granuloma uveitis. Conservative treatment with topical steroids in cases of AC granuloma and regional ocular steroids injections in cases of intermediate uveitis, along with oral steroids for a duration of 8–12 weeks. (9) Alternatively,



interventional therapy via surgery such as anterior chamber wash and pars plana vitrectomy (PPV). According to a recent study, early pars plana vitrectomy with proper shaving of the ciliary body granuloma helps control the inflammation and prevent the eye from progressing to TRD. (5)

In this study, aqueous and vitreous fluid samples will be obtained from patients presenting with anterior and intermediate uveitis, respectively, who have not responded to conservative treatment and require surgical management by AC wash or pars plana vitrectomy (PPV). Samples obtained will be subjected to DNA extraction followed by selective polymerase chain reaction (PCR) using trematode-specific primers targeting highly conserved genomic regions. In some cases, the amplified PCR products will be further analyzed by sequencing and BLAST analysis for confirmation and species identification.

6. Objectives:

To identify the causative organism of presumed trematodal induced uveitis in Egypt through genetic analysis of ocular fluid samples

7. Study Design:

Descriptive: - Survey (cross sectional) ☐

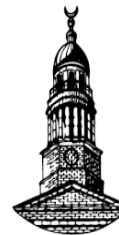
- Qualitative ☐

- Analytic: - Observational: - Case-control study ☐

- Cross sectional analytic study ☒

- Cohort (Longitudinal) study ☐

- Experimental: - Randomized Clinical Trial ☐



Phase:

- Non-randomized clinical trial ☐
- Animal study ☐
- Cellular study ☐

- Others: Please describe:

8. Study Methods

- Population of study:

- Patients with PTIGU of any age presenting to the Kasr Al-Aini Uveitis and Ocular Immunology Clinic who are scheduled to undergo AC wash or pars plana vitrectomy will be included in the study. The diagnosis of PTIGU will be based on the following criteria: 1) history of fresh canal water contact in one of the branches of the River Nile prior to the onset of uveitis, 2) signs of anterior uveitis with the presence of typical AC granuloma 3) signs of intermediate uveitis with the confirmation of CB granuloma by ultrasound biomicroscopy (UBM), 4) absence of clinical signs or work-up suggestive of any other cause of granulomatous Uveitis. 5) Failure of medical treatment by steroids in the form of persistent anterior segment flare and cells $>+1$ or posterior segment inflammation ($>$ Grade 2 vitritis).

- Study location:

Kasr Al-Aini Uveitis and Ocular Immunology Clinic, Cairo University Hospitals.



- **Inclusion criteria:**

Patients with Presumed Trematode-Induced Uveitis scheduled for AC wash or pars plana vitrectomy of any age.

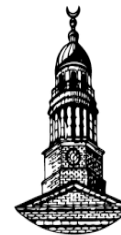
- **Exclusion criteria:**

- Causes of granulomatous uveitis other than trematodal infection.
- Inconclusive UBM findings.
- Ocular trauma.
- Previous ocular surgeries including (anterior chamber wash for AC granulomas).

- **Methodology in detail:**

For all included patients, we will retrieve the age, gender, eye laterality, duration of disease, and the treatment received (topical, regional, and systemic). Careful review of systems and thorough investigations will be done as guided by clinical presentation (to exclude other causes of granulomatous uveitis). These investigations may be in the form of complete blood picture, stool and urine analysis, fluorescent treponemal antibody absorbent test, angiotensin-converting enzyme, tuberculin skin testing, toxocara antibodies, toxoplasma antibodies, and chest X-ray.

All patients will undergo thorough clinical examination including corrected distance visual acuity (CDVA), intraocular pressure (IOP) measurement, and ophthalmic findings with slit-lamp examination will be collected including the AC with associated granulomas, iris status regarding presence of synechiae, gonioscopic findings, lens status and vitreous inflammatory grades according to the

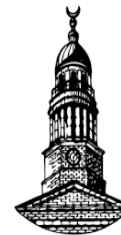


Standardized Uveitis Nomenclature (SUN) classification. Dilated fundus examination with slit-lamp biomicroscopy and indirect ophthalmoscopy with scleral indentation will be explored to document the presence of retinal detachment, macula status, and grades of proliferative vitreoretinopathy (PVR). Also, B scan ultrasonography (US) will be done if media opacities precluded fundus examination. Ultrasonographic biomicroscopy (UBM) will be done to determine the presence and location of ciliary body granuloma.

All surgeries will be done under general anesthesia by the same experienced posterior segment surgeon. Aqueous fluid will be aspirated by using aseptic precautions and a 25-gauge needle passing through the limbus. In cases of PPV, any posterior synechiae will be released and the pupil will be dilated with the help of iris hooks whenever necessary. Lensectomy without IOL implantation will be done through anterior or posterior approach.

PPV will be done using a 23-gauge cannula system and cannulae will be inserted at sites away from the CB granuloma location pre-determined by UBM. Vitreous fluid samples of 100-200 μ l will be obtained then debulking of the granuloma will be done as much as possible, taking care not to injure the CB as the granuloma is always amalgamated with it. Diathermy will be applied to the remaining parts of the granuloma as well as any bleeding points. All patients will be left aphakic with a plan for secondary implantation later when the ocular inflammation subsides.

All eyes will receive topical antibiotics with topical prednisolone acetate 1% eye drops given every 2 hours for the first 48 hours followed by 5 times daily to be tapered according to AC reaction in addition to antibiotic combined with steroid ointment at bedtime.

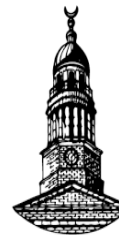


Samples will be stored in sterile Epindorf tubes at -80°C . DNA will be extracted from the samples using DNeasy tissue kit (Qiagen, Hilden, Germany) according to manufacturer's.

A primer pair, CO1F (5-GATCGTA AATTTGGATACTGC-3) and CO1R (5-CCAACCATAAACATATGATG-3) targeting trematodal mitochondrial cytochrome c oxidase subunit 1 (CO1) gene will be used. PCR will be made in 25 μL reaction containing 12.5 μL MyTaq HS red Master Mix (Bioline, England), 0.5 μM of each primer and 0.5 μL DNA extract. PCR conditions will include initial denaturation at 95°C for 4 min followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 54°C for 15 s and extension at 72°C for 30 s. PCR products will then be examined on 1.5% agarose gel stained with ethidium bromide to detect the expected 250 bp amplified product. In samples undergoing sequencing, the amplified products will be loaded on the gel and purified using the DNA Purification Kit (Promega, Madison, WI, USA). The cyclic sequencing reaction will be performed. Samples will be denatured at 96°C for 2 min and then cycled 28 times at 96°C for 10 s, 52°C for 10 s, and 60°C for 4 min. Unincorporated nucleotides will be removed by using sodium carbonate, 125 mmol/L EDTA, and absolute alcohol. HI-Di Formamide (Applied Biosystems) will be used to stabilize the single-strand template before preforming sequencing. Sequences will be analyzed by using BLAST for species-level identification.

Post-operative follow-up data:

During post-operative visits, a complete ophthalmological examination documenting any postoperative complications and the course of inflammation will be done on the first post-operative day, weekly for 1 month, then monthly thereafter for 6 months. Secondary implantation will be scheduled for 3 months following vitrectomy in cases of controlled inflammation. In silicone oil-filled globes (SOFG)



with stable attached retinæ, silicone oil removal will be performed 3 months after the initial surgery.

- **Intervention:**

- ☒ Diagnostic intervention: To analyze the ocular fluid in presumed trematode-induced uveitis.
- ☐ Therapeutic intervention (please describe):
- ☐ No intervention

- **Does the research involve?**

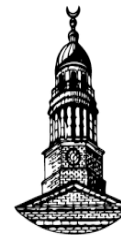
- ☒ Human participants
- ☐ Biological samples/Tissues
- ☐ Identifiable private data/Information

- **Type of consent of study participants:**

- ☒ Written consent
- ☐ Oral consent
- ☐ No consent needed (Please justify)

- **Potential risks:**

- 1- Minimal discomfort from direct eye contact with UBM.



- 2- Complications of AC wash including hyphema, iris or lens trauma
- 3- Complications of PPV including hemorrhages, retinal detachment or elevated intraocular pressure.

Confidentiality of data: -

Patient consent will be obtained, and all information will remain confidential, - protecting patients' anonymity and rights. All participants will be coded, and data entry will be using these codes. All data of the patients will be saved in secure files.

9- Study outcomes:

- Primary outcomes

- 1- To detect trematodal DNA in the ocular fluid of presumed trematode-induced uveitis by using PCR
- 2- To identify the organism responsible for trematode-induced uveitis through DNA sequence analysis of genetic material extracted from ocular fluid.

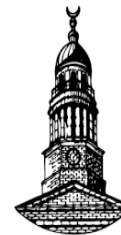
- Secondary outcome parameters (other outcomes to be assessed)

- 1- To compare the diagnostic yield of aqueous and vitreous fluid samples for molecular detection of trematode DNA.

10- Sample size

Sample size calculated based on the trematodal DNA in the ocular fluid of trematode-induced uveitis by using PCR. Arya et al., 2016 reported that 13 out of 42 granuloma samples were positive for trematode DNA (31%). (7)

Sample size calculated using G*power 3.1.9.7. software. Exact test,



proportion: difference from constant (binomial test, one sample case). The input parameters are: $p_1 = 0.01$ (normal population), $p_2 = 0.31$, one tail test, effect size $g = 0.3$, $\alpha = 0.05$, power = 0.95.

The calculated sample size is 32. After addition of 20% dropout, the final sample size equals 40 patients.

Sampling technique:

A convenient sample of patients with the inclusion and exclusion criteria will be sampled and assigned into the study till reaching total sample size calculated.

11- Statistical analysis

Data analysis packages will be SPSS version 21. Qualitative data will be presented by number and percentage, quantitative data will be presented by mean, standard deviation, median and interquartile range.

Parametric and non-parametric tests of significant will be done (chi square, student t test and Mann whitney test).

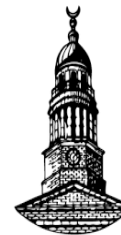
ROC curve analysis will be done. Correlation analysis tests and regression will be done.

Level of significance was set at p equal to or below 0.05.

12- Source of funding: (Please include source of funding even if self funding)

- Faculty of Medicine, Cairo University
- Other sources: ☐

Please specify: **Self-funding**

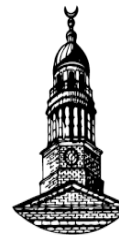


13- Time plan:

- When to start? After gaining the approval of the scientific committee, ethical committee and the faculty.
- When expected to finish? After data collection which will take around 6 months from gaining the approval of the scientific committee, ethical committee and the faculty.
- When to publish? After completion of the study

14- References:

1. El Nokrashy, A., Abou Samra, W., Sobeih, D. *et al.* Treatment of presumed trematode-induced granulomatous anterior uveitis among children in rural areas of Egypt. *Eye* 33, 1525–1533 (2019).
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7. Arya LK, Rathinam SR, Lalitha P, Kim UR, Ghatani S, Tandon V. Trematode fluke *Procerovum varium* as cause of ocular inflammation in children, South India. *Emerg Infect Dis*. 2016;22(2):192–200.
8. Rathinam SR, Arya LK, Usha KR, Prajna L, Tandon V. Novel etiological agent: molecular evidence for trematode-induced anterior uveitis in children. *Arch Ophthalmol*. 2012;130:1481–4.
9. Amin RM, Radwan AE, Goweida MB, El Goweini HF, Bedda AM, Lotfy WM, et al. Management of presumed trematode induced granulomatous uveitis in pediatric patients. *Jpn J Ophthalmol*. 2019;63(1):119–25.
10. El Nokrashy A, Abou Samra W, Sobeih D, Lamin A, Hashish A, Tarshouby S, et al. Treatment of presumed trematode-induced granulomatous anterior uveitis among children in rural areas of Egypt. *Eye*. 2019;33(10):1525–33.
11. Badir, G.. (1946). SCHISTOSOMIASIS OF THE CONJUNCTIVA. *British Journal of Ophthalmology*, 30(4), 215–221.

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- 2- For choice brackets, please just use the fill in function in word