Japanese Journal of Ophthalmology (2019) 63:119–125
https://doi.org/10.1007/s10384-018-0632-3

THE OFFICIAL INTERNATIONAL JOURNAL OF THE JAPANESE OPHTHALMOLOGICAL SOCIETY

CLINICAL INVESTIGATION

Management of presumed trematode induced granulomatous uveitis in pediatric patients

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Received: 19 March 2018 / Accepted: 30 August 2018 / Published online: 2 November 2018

Abstract

Purpose To outline the management of newly identified trematode induced uveitis in pediatric patients

Study design Prospective interventional case series

Methods Patients with distinctive uveitis were recruited to either receive steroid monotherapy or undergo surgical excision of the inflammatory lesions based on a scoring system. Outcome measures included best corrected visual acuity (BCVA), intraocular inflammatory activity, and incidence of ophthalmic complications

Results 170 patients (174 eyes) were recruited. Mean age was 11.1 years. Mean initial decimal BCVA (±SD) was 0.58 (±0.31). Of 116 eyes with disease scores <5, 109 were treated effectively with steroids (93.97%). Surgical excision was offered to 58 patients and proved curative in the treated eyes. Protracted inflammation with persistence of the granulomas was noted in 5 patients refusing surgery. Mean follow up period was 21.5 months. Mean final BCVA was 0.69 (±0.27). A significant change in BCVA was noted (p=0.002). There has not been a need for retreatment in any of the study patients, who were also given instructions on evading exposure to fresh water habitats. Larger lesions, mixed disease morphology, older age at presentation were associated with higher rates of ophthalmic complications and vision loss

Conclusion A novel waterborne trematode inducing uveitis has been identified in Egypt. A favorable response to steroid monotherapy is demonstrated in low grade disease, while surgical excision was found to be curative in patients with larger lesions or those showing suboptimal response to medical treatment

Keywords Infections · Granulomatous uveitis · Treatment · River Nile · Egypt

Introduction

Infectious causes of uveitis are particularly common in developing countries, with various infectious agents increasingly being identified as novel causes of intraocular inflammation [1–4]. It is estimated that in the USA 10% of cases of blindness are attributed to uveitis, while in Africa, a region where uveitis is predominantly attributed to ophthalmic infections it is 24% [5, 6]. Several helminthes are reported to cause various ophthalmic manifestations in humans [3, 4, 7–9]. These flukes have complex life cycles involving various fresh water snails, aquatic birds, and mammals, and can under rare circumstances invade the eye eliciting a robust immune response [10]. In these cases, blindness will oftentimes result from protracted intraocular inflammation, and prompt diagnosis coupled with appropriate management remains key to the prevention of further tissue damage and vision loss.

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In a recent epidemiologic report from Egypt, we have identified a distinctive pattern of intraocular inflammation amongst a group of pediatric patients [11]. The disease has been shown to affect children in rural villages after swimming in the fresh waters of the River Nile, causing anterior uveitis with white anterior chamber (AC) nodules that eventually evolve into retrocorneal scars. The lesions were only occasionally associated with subconjunctival nodules and dense vitritis. Further analysis of excised intracameral granulomas has revealed ocular parasitosis, where molecular evidence of a digenetic trematode isolated in 42.8% of the samples using conventional polymerase chain reaction (PCR) targeting mitochondrial cytochrome c oxidase subunit 1 (COI); a barcode for parasitic Platyhelminthes [12]. A similar presentation in children from South India is reported, and further DNA subunit analysis had incriminated yet another digenetic trematode; procerovum varium, as the cause of this peculiar presentation [13]. No molecular or histopathologic evidence of tuberculous mycobacteria (TB) or fungi has been detected in the collected ocular samples [4, 14].

The uveitis in these cases is exceptional, and is presumably the result of incidental human infection under particular environmental circumstances. The inflammation is notably chronic and persistent, and vision is oftentimes lost to extensive AC scarring or phthisis [4, 12]. It seems that the global extent of these waterborne pediatric parasitic granulomas will continue to expand, as more parasitic agents are being identified as potential causes of similar outbreaks, and a challenge is posed to local ophthalmologists [15]. This prospective study was conducted enrolling more children from our locality to highlight clinical considerations for the management of this peculiar pathology.

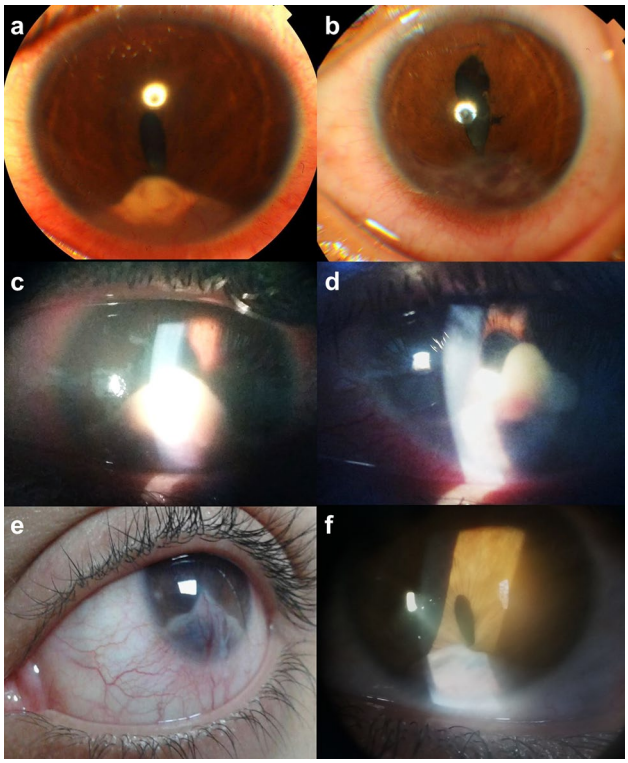

Fig. 1 **a** and **b** Sequential anterior segment photographs of a 6 year old boy with left eye retrocorneal membrane in addition to an anterior chamber (AC) inflammatory nodule before and after surgical excision with pupilloplasty. **c** Shows a mixed type lesion with a disease score of 8, treated with topical steroids in a patient who has refused surgery. **d** Follow up after 12 weeks reveals persistence of the AC nodule and protracted intraocular inflammation. **e** Retrocorneal scarring in a patient who has refused surgical intervention at presentation for a disease score of 5. **f** Corectopia in an 11 year old from Northern Egypt as a sequel of presumed trematodal granulomatous anterior uveitis

Materials and methods

The study has followed the tenets of the Declaration of Helsinki and approvals of the ethics committees in all the recruiting institutions were obtained. Patients with distinctive granulomatous uveitis attending the study referral centers were recruited, including patients from our previous series [12]. All of these patients presented with granulomatous anterior uveitis associated with one or more white nodules in the AC and/or a retrocorneal membrane following bathing in the local waters (Fig. 1). Patients with previous history of ophthalmic trauma or surgery were excluded from the study. Demographic data including age, gender, place of residence were recorded. All patients were referred to a local physician for full examination to exclude any other associated systemic granulomatous disease as well as full blood counts, urine, and stool sample collection.

Decimal Best corrected visual acuity (BCVA) and ophthalmic findings upon slit-lamp examination were recorded including AC inflammatory grades (SUN Grading Scheme) [16]. A disease scoring system was proposed based on the size of the AC lesions [4, 17], grade of associated AC inflammation, and ocular complications noted at the time of presentation (Table 1). Patients with low grade disease (score <5) were started on hourly topical prednisolone acetate 1% together with 1 mg/kg oral prednisone, then reviewed weekly, tapering treatment according to response over a maximum period of 12 weeks. The AC lesions were surgically removed whenever the disease score at presentation was ≥ 5, or when persistent active AC inflammation was noted after 12 weeks of steroid monotherapy. Following informed patient/guardian consent, the AC nodules were surgically removed through a paracentesis stab incision and a nylon suture was placed if needed. In selected samples, formalin-fixed paraffin embedded sections of ocular tissue were stained with hematoxylin and eosin (H and E) for further

Table 1 Disease Scores at presentation for Parasitic Granulomatous Uveitis

Parameter	Range	Score
Diameter of AC lesion	<3 mm	1
	3-5 mm	2
	>5 mm	3
Grade of AC inflammation*	0.5+ to 1+	1
	2+ to 3+	2
	4+	3
Complications at presentation	None	0
	Retrocorneal scar or Corectopia	1
	Cataract	2
	Glaucomatous optic atrophy	3

Eyes with scores< 5 were started on steroid therapy. Patients with severity scores ≥ 5 at presentation were advised for surgical excision of the AC lesions

*Based on SUN Grading Scheme for AC Cells

histopathologic examination, while DNA was extracted using DNeasy blood and tissue kit (Qiagen) according to manufacturer's instructions. The primer pair CF (5-GAT CGTAAATTTGGA/TACTGC-3) and CR (5-CCAACC ATAAACATATGATG-3) targeting digenetic trematodal mitochondrial cytochrome COI gene was used in this study [18]. PCR was made in 25 µL reaction containing 12.5 µL MyTaq HS red Master Mix (Bioline), 0.5 µM of each primer, and 0.5 µL DNA extract. PCR conditions included initial denaturation at 95°C for 4 minutes followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 54°C for 15 seconds and extension at 72°C for 30 seconds. PCR products were examined on 1.5% agarose gel stained with ethidium bromide. Another run using the 28S r DNA primer employed by Rathinam et al. for heterophyidae trematodes AP103 F 5'AGAGCGCAGCCAACTGTGTGA3' and AP103 R 5'TGCCACGTCCTAGCATCAGCC3 was also performed [14]. All patients were reviewed at monthly intervals for at least one year thereafter, and BCVA, sequelae related to the uveitis or its management, as well as AC inflammatory activity were recorded.

Statistical analysis

Commercially available statistical software (IBM-SPSS version 22 for Windows; IBM Inc.) was used for data analysis. Variables were presented as counts, percentages and means±SD. The frequency of categorical observations in different groups was compared by Chi-Square Test (χ^2). Mann–Whitney U and Kruskal–Wallis tests were used to compare mean ranks of visual acuity among different study groups. *p* value of less than 0.05 was considered statistically significant.

Results

Over the study period from March 2014 through February 2017, 170 patients (174 eyes) were recruited for this study including 152 boys and 18 girls. All patients were residents from villages along the River Nile basin in Egypt and all reported swimming in local river waters prior to presentation. None of the patients had a systems' review or work up suggestive of active systemic granulomatous disease, nor did they report symptoms of dysuria or bloody stools. Mild eosinophilia was reported in 12% of the cases, while no hematuria or parasitic ova were found in the collected urine or stool samples. Patients' ages ranged between 5 to 20 years with a mean of 11.1 ± 2.86 years. One hundred and sixty six patients had unilateral uveitis while only four had bilateral disease (2.4%). The mean follow up period for the study cases was 21.5 months (range 12-48 months).

Following slit-lamp examination, the diameters of the lesions were found to be less than 3mm in 107 eyes, 3 to 5mm in 23 eyes, and more than 5mm in 24 eyes, all associated with different grades (0.5+ to 4+) of active AC inflammation. Complications such as cataract, glaucoma, corectopia, phthisis, or retrocorneal scarring straddling the pupillary axis were encountered in 16% of study eyes (17, 7, 5, 3, and 2 cases respectively). 116 eyes were described as having low grade disease (scores <5) while the remaining 58 eyes were given scores of ≥ 5. The former group was treated with oral and topical steroid therapy and a favorable response with resolution of the uveitis and disappearance of the AC nodules was recorded in 109 eyes (93.7%). On their final review, an improvement in BCVA was reported in twelve eyes, while 93 eyes were found to have the initial vision. Deterioration by one or more lines was reported in four eyes due to the development of secondary cataracts. All eyes sustained a remission of the uveitis throughout follow up.

Pathological lesions were surgically removed in 56 eyes including 49 eyes of patients with uveitis scores ≥ 5 who consented to surgical treatment, and seven eyes where there was incomplete resolution of the inflammation following 12 weeks of steroid treatment. Nine patients with high disease scores at presentation refused surgical intervention. Histopathological evaluation of H and E stained sections of the excised lesions showed a granulomatous reaction formed predominantly of epithelioid cells admixed with lymphocytes and few eosinophils. Trematodal COI DNA was identified in five of thirteen AC samples whereas none of the samples tested positive for a heterophyide (Fig. 2). On their final visit, 40 of the 56 patients treated surgically showed improvement in BCVA by one or more lines (76.9%). Twelve patients retained their initial vision, while four cases were lost to

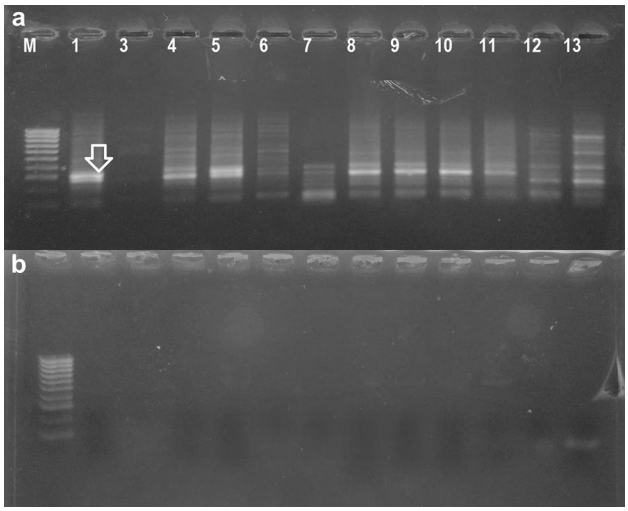

Fig. 2 **a** Shows samples from 1-13 (except 2) on 1.5% agarose gel to detect PCR amplified product using the proposed trematodal COI primers. The arrow indicates the band. **b** None of the tested samples revealed the heterophyide subunit DNA band

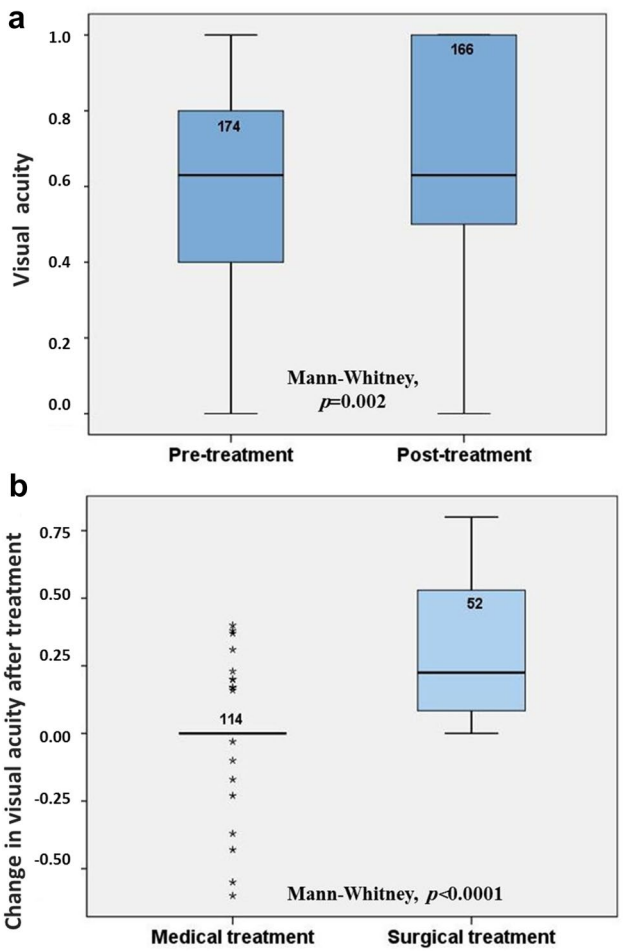

Fig. 3 Box and whiskers plots showing initial and final decimal best corrected visual acuity after treatment of the distinctive granulomatous uveitis in **a** and change of visual acuity after medical compared to surgical treatment of the investigated cases in **b**. Horizontal bars indicate median visual acuity, boxes represent 50th percentiles, whiskers represent range of data, and stars refer to outliers, while numbers refer to total number of eyes in each group

follow up. Neither deterioration in BCVA nor recurrence of intraocular inflammation were reported after surgical treatment (p <0.0001). Hyphema was identified in four patients on the first postoperative day, but no other sequelae related to the surgery were reported. Prolonged courses of topical steroid therapy were given to patients who refused surgery (n=9 eyes) of which five returned for review. Persistent intraocular inflammation was noted in all five cases throughout follow up with persistence of the AC nodules (Fig. 1). Deterioration in BCVA was reported in four eyes due to visually significant retrocorneal scarring (n=2), complicated cataract (n=1), and glaucomatous optic atrophy (n=1). The initial and final BCVA of the study patients are summarized in Figure 3.

We note that patients older than 10 years of age at the time of diagnosis were found to have a significantly reduced initial visual acuity and a higher ocular complications rate (p=0.007 and 0.079 respectively). In addition, nodules of diameter >5mm, mixed morphology with AC nodule adjacent to a retrocorneal membrane, severe AC inflammation, and high disease severity score at presentation were all significantly associated with a high incidence of ophthalmic complications and vision loss (Table 2).

Discussion

The first description of an eye fluke was in Egypt in 1899, when the trematode Philophthalmus palpebrarum Looss was identified in the conjunctival sac of a local bird [19, 20]. A variety of mammals, including humans, have been reported thereafter to develop conjunctival philophthalmosis which can result in corneal scarring and vision loss [21–24]. Other eye flukes of fish eating birds have also been reported to infect humans, and one has recently been implicated as a cause of intraocular inflammation and blindness in children from South India [13]. The disease appears to have long been recognized, yet has gone undiagnosed or possibly under reported in endemic areas [17]. Amongst Egyptian ophthalmologists it has been referred to over the years as 'pearl's tumor', and the notion that these intracameral lesions could be TB induced 'conglomerate tubercles' has previously been disputed [17].

The incidence of these ophthalmic infections appears to be notably high, where up to 30% of pediatric uveitis cases both in Egypt and South India have been attributed to these parasitic infections [4, 11]. Characteristics of the ocular disease are identical, and although no definite foreign

Table 2 Decimal best corrected visual acuity (BCVA) at presentation and incidence of ocular complications in 170 patients affected by trematodal granulomatous uveitis from Egypt

Parameter	BCVA at presentation		Complications at presentation		
	Mean (SD)	*p* value	Present (n)	Absent (n)	*p* value
Age					
≤10 years	0.64 (0.30)	0.007*	8	68	0.08‡
>10 years	0.52 (0.31)		20	78	
Sex					
Male	0.58 (0.31)	0.679*	28	128	0.18‡
Female	0.54 (0.29)		1	17	
Involved Eye					
Right	0.68 (0.28)	<0.001*	15	80	0.90‡
Left	0.46 (0.31)		13	66	
Clinical Pattern					
Nodular	0.64 (0.28)	<0.001†	17	121	0.03‡
Membranous	0.54 (0.32)		5	11	
Mixed	0.14 (0.12)		6	14	
Size of the lesion					
<3mm	0.73 (0.23)	<0.001†	7	100	<0.001‡
3-5mm	0.42 (0.26)		13	30	
>5mm	0.16 (0.14)		8	16	
AC Inflammation					
SUN 0.5+	0.73 (0.23)	<0.001†	7	98	<0.001‡
SUN 1-2+	0.43 (0.23)		10	7	
SUN 3-4+	0.32 (0.28)		11	41	
Disease Severity					
Low grade (score <5)	0.72 (0.23)	<0.001*	7	109	<0.001‡
High grade (score ≥5)	0.29 (0.25)		21	37	

*Mann-Whitney U test, †Kruskal-Wallis, ‡Chi-square test

bodies have been identified upon microscopic examination of the AC lesions in either series, molecular DNA of digenean trematodes has been isolated [12–14]. This subclass of Platyhelminthes comprises thousands of species; some yet to be described, and typically requires a mollusk as their first intermediate host [25]. These ultimately release large numbers of cercarial larvae which parasitize second intermediate or definitive vertebrate hosts after oral consumption, or actively through skin or mucosa penetration as they become exposed to fresh water repertoires. Procerovum varium is a digenean fluke of fish eating birds of the family heterophyidae, found in the waters of many countries of the Far East, and although other genera of this family are known to the Egyptian fresh water fauna, none of our samples have tested positive for a heterophyide [26, 27]. It is also unclear why molecular testing in some of the samples was negative, yet the use of conventional rather than real-time PCR in our study; the latter being a more sensitive method for nucleic acid detection in small samples, could be a contributing factor [28]. Histopathologic analysis of thirteen of the associated subconjunctival nodules with these presentations has revealed the tegument of a worm in four [14]. Similar presentations have historically been described as allergic conjunctival nodules, and despite a proposed association with parasitic infections, histologic evidence of helminths has only rarely been identified [29]. Interestingly though, excision of these lesions was found to be curative [29–31]. It could be that a temporal relation between the time of ophthalmic infection and that of tissue biopsy is affecting the chance of parasite cuticle detection. This, coupled with a potent local inflammatory response, could explain the lack of direct histopathologic evidence of fluke debris in intraocular samples.

A favorable response to steroid treatment has been demonstrated in this series where cases with low grade disease have been treated with steroid monotherapy. Symptomatic children from South India have also responded to corticosteroid treatment with resolution of the inflammation and scarring of the AC nodules, possibly providing further evidence against presumed TB or fungal etiology [4, 17]. We also conclude that surgical excision is a safe treatment option, and is possibly preferred for larger lesions with severe uveitis or active small lesions resistant to medical treatment to remove debris from within the eye. Treatment appears to be curative, particularly with instructions on evading bathing in the river ponds, and there has not been a need for retreatment

in any of the study cases. We note that certain clinical features including disease type at presentation, severity of AC inflammation, and size of the lesions appear to be significant risk factors for vision loss. In addition, a direct relationship between age at presentation and disease severity was noticed, and could possibly be attributed to longer duration of active ophthalmic disease or age-related alterations of the immune response [32, 33].

None of the patients in this series has received antimicrobial treatments, and to the best of our knowledge, the value of praziquantel treatment in these cases remains elusive; whilst these medications are known to act on biochemical metabolic pathways in adult flukes [34], the ophthalmic lesions appear to be reactive granulomata to residual larval debris [3, 4, 12, 17]. Moreover, none of the children had systemic findings suggestive of florid parasitosis to justify systemic treatment. The exact mode of ophthalmic infection in this peculiar pathology remains unclear, yet knowing the invasiveness of helminthic larvae, one can postulate for a local route involving limbal or uveoscleral channels, or alternately, blood borne infection following oral mucosa penetration [25, 35].

Interestingly, similar ophthalmic nodules are described in a series of children from the Amazonia in Brazil following similar environmental exposures [15]. 78% of these patients have responded favorably to oral steroid therapy whereas further intervention including excision of the nodules was necessary in the remaining cases, and it seems likely that the global scope of these outbreaks will continue to expand with various parasites, both protozoans and helminths being identified as possible culprits. Advanced molecular techniques are imperative to further identify parasitic species, as it is a challenge to attempt this solely on histopathology of ophthalmic tissue [36]. Preventive measures need to be in effect in known endemic areas, and these can entail public health education, evading exposure to the host fresh water habitats, as well as conducting local environmental surveys on water repertoires to identify and eliminate snail reservoirs from the fresh water fauna.

Conflicts of interest R. M. Amin, None; A. E. Radwan, None; M. B. Goweida, None; H. F. E-. Goweini, None; A. M. Bedda, None; W. M. Lotfy, None; A. RH. Ahmed, None.

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