DOI: 10.5958/2319-5886.2014.00391.9

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 3 Issue 3 Coden: IJMRHS Copyright @2014 ISSN: 2319-5886

Received: 12th Mar 2014 Revised: 26th Apr 2014 Accepted: 17th May 2014

Research Article

REAL TIME POLYMERASE CHAIN REACTION (RT-PCR) FOR *MYCOBACTERIUM TUBERCULOSIS* IN SERPIGINOUS CHOROIDITIS- A STUDY OF 29 CASES

*****Radha Annamalai[1], Jyotirmay Biswas[2], S Sudharshan [3], R Gayathri [4],K Lily Therese[5], Viswanathan S[6], Namitha Bhuvaneswari[7]

[1]Associate Professor, Department of Ophthalmology, Sri Ramachandra University, Porur, Chennai,India
[2]Director of Uvea & Ocular Pathology Department, Sankara Nethralaya, Chennai,India
[3]Consultant- Department of Uvea, Sankara Nethralaya, Chennai,India
[4]Postdoctoral fellow, &[5]Senior Professor and HOD, L & T Microbiology Research Centre, Vision Research Foundation, KNBIRVO Building 41, College Road, Chennai - 600 006
[6]Professor of Ophthalmology, Muthukumaran Medical College, Chennai, India
[7]Director and Professor of Ophthalmology, Regional Institute of Ophthalmology, Chennai, India

* Corresponding author email: drradhaannamalai@yahoo.co.in

ABSTRACT

Purpose: A study of real time Polymerase Chain Reaction for *Mycobacterium tuberculosis* (*M. tuberculosis*) DNA in 29 cases of active serpiginous choroiditis. Design: Case control study. **Methods:** DNA extraction from the aqueous humor was carried out using QIAMP DNA extraction kit. Real- time Polymerase Chain reaction (RT-PCR) for MTB was carried out using Genosen's Mtb complex quantitative Real time PCR kit. All patients were also subjected to complete blood count, venereal disease research laboratory test, chest radiograph, QuantiFERON TB Gold test on the blood and polymerase chain reaction on a sample of aqueous humor. **Results:** Aqueous aspirate showed copies of mycobacterium tuberculosis DNA in one out of twenty nine cases of serpiginous choroiditis. Direct smear and culture for mycobacteria was negative in all cases. **Conclusion:** RT-PCR identifies MTB DNA in suspected latent tuberculosis in serpiginous choroiditis with high specificity. Serpiginous choroiditis and multifocal choroiditis due to tuberculosis may resemble each other clinically but have distinct clinical features which can be confirmed by real time polymerase chain reaction performed on the aqueous humor The association between serpiginous choroiditis and tuberculosis would be a chance association or if present a rare association.

Keywords: Real-time polymerase chain reaction *(RT-PCR)*, Serpiginous choroiditis, Ampiginous choroiditis, tuberculosis, QuantiFERON TB Gold test

INTRODUCTION

Serpiginous choroiditis is a chronicprogressiveinflammatorydisease. It is rare, usually bilateral but asymmetrical and is seen between the ages of 30 and 70 years. It begins around the optic nerve in most eyes, advancing centrifugally by recurrences to the mid periphery in an irregular serpentine fashion. Active serpiginous choroiditis characterized by greyish-yellow, cream-colored lesions at the level of retinal pigment epithelium (RPE) with overlying retinal edema.[1] In some eyes, however, the macula is affected initially without preceding peripapillary activity, a variant known as macular serpiginous choroiditis.[2] In addition, occasionally patients present with involvement of

peripheral retina as the primary site of affection. New recurrent lesions occur at the border of old inactive lesions and frequently spread to the periphery, commonly involving a new and contiguous area of the fundus. Various aetiologies such as autoimmunity[3], infection[4], degeneration and vasculopathy have been assumed to cause serpiginous choroiditis. Irreversible profound visual loss can result due to complications such as chorioretinal atrophy, scarring and choroidal neovascular membranes. We performed a study on 29 eyes of 27 patients with serpiginous choroiditis with suspected latent tuberculosis (TB) and found that in one case *Mycobacterium tuberculosis* (*M .tuberculosis*)DNA was detected in aqueous humor aspirate by real-time polymerase chain reaction (RT-PCR).

MATERIAL & METHOD

The study was conducted in a tertiary referral hospital in India. Prior to the study ethics committee clearance was obtained. Inclusion criteria comprised all patients with serpiginous choroiditis and multifocal choroiditis which were suspicious for tuberculosis. Patients with other causes of posterior uveitis and those where the serpiginous choroiditis was inactive or healed were excluded from the study. The aqueous aspirate was obtained from 29 eyes of 27 patients and 27 controls during cataract surgery. Examination was performed on all controls using slit lamp and biomicroscopy. They were healthy patients with no evidence of intraocular inflammation or uveitis. An anterior chamber tap was performed under aseptic precautions using povidone iodine and 0.1ml of aqueous humor sample was sent immediately to the microbiology department. Complete blood count, QuantiFERON TB Gold test and high resolution chest tomography (HRCT) and polymerase chain reaction on the aqueous humor sample were performed in all the cases. DNA extraction from the aqueous humor was carried out using a QIAMP DNA extraction kit (QIAGEN, Germany). Real time Polymerase Chain reaction (RT-PCR) for *M. tuberculosis* was carried out using Genosen's MTB complex (Netherlands) quantitative Real time PCR kit. RT-PCR for quantitation of MTB DNA was carried out as a 25 µl reaction, using 12 µl of MTB complex super mix R1, 2.5 µl of Magnesium solution R2 and 0.5 µl of Internal control IC 1 R3 and 10 µl of aqueous humor DNA. The amplification was carried out at an initial denaturation at 95 º C for 10 minutes, followed by 45 cycles of 95 º C for 15 seconds, 60 º C for 20 seconds, 72 º C for 15 seconds. The quantitation analysis for the internal control and *M. tuberculosis* was carried out using JOE (yellow) and FAM (green) channel. The copy number of *M. tuberculosis* was expressed in copies per ml of DNA

RESULTS

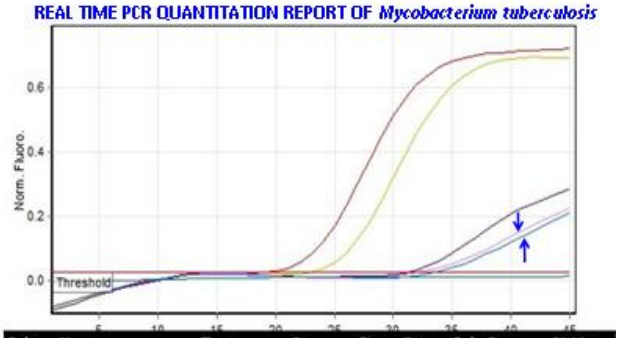
Aqueous aspirate showed copies of *M. tuberculosis* DNA in one out of twenty nine cases of serpiginous choroiditis. Direct smear and culture for mycobacteria was negative in all cases.

RT PCR was positive in one case which is described below:

A 38 year old Asian Indian male presented to the uveitis clinic with a history of gradual diminishing vision for one month. He was being treated with systemic corticosteroids prescribed elsewhere. Ocular examination revealed a best-corrected visual acuity of 6/60, N24 in the right eye and 6/6, N6 in the left eye. Slit lamp examination revealed no aqueous cells or flare and 1+ vitreous cell in the right eye. The left eye was normal. Intraocular pressure was 12 mmHg in both eyes. Fundus examination in the right eye revealed active choroiditis with geographic borders and a clinical diagnosis of serpiginous choroiditis was made (Figure 1). Chest X Ray and ESR were normal. Tuberculin skin test was negative. An anterior chamber tap was done in the right eye and the aspirate was subjected to direct smear, culture, analysis by polymerase chain reaction (PCR) and RT-PCR for *M. tuberculosis* genome. RT-PCR performed on his aqueous aspirate showed 14,781 copies of *M. tuberculosis* DNA (Figure 2). Direct smear and culture for *M. tuberculosis* were negative. He had no symptoms of systemic tuberculosis (TB) but QuantiFERON TB Gold test done on his blood sample was positive. The patient was started on antituberculous treatment and corticosteroids under supervision of an infectious diseases specialist. Follow up after 2 months showed that the lesions had resolved (Figure 3) and RT-PCR of aqueous was negative for *M. tuberculosis* genome (Figure4). Visual acuity had improved to 6/24, N12 in the right eye. Control samples from 27 cases of anterior chamber aspirate of patients without uveitis undergoing phacoemulsification were subjected to

RT- PCR. All were negative for *M. tuberculosis*(Figure 5).

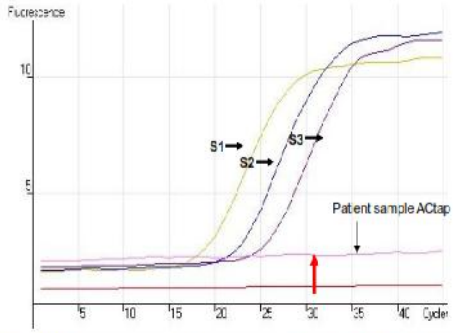
Fig1: Active serpiginous choroiditis



Colour	Name	Type	Ct	Given Conc (copies/ml)	Calc Conc (copies/ml)	% Var
	S1	Standard	19.06	25,000,000	24,970,236	0.1%
	S2	Standard	23.13	2,500,000	2,504,471	0.2%
	S4	Standard	31.29	25,000	24,985	0.1%
	VRF 1406/13 10	Unknown	32.22		14,781	
	VRF 1406/13 15	Unknown	33.46		7,364	
	NC	NTC				

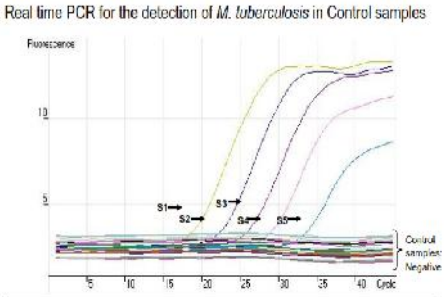
Fig 2: Positive results of real time PCR of Aqueous aspirate for *M. tuberculosis*

Fig 3: Resolved serpiginous choroiditis



Colour	Name	Type	Ct	Given Conc (IU/ml)	Calc Conc (IU/ml)
	NC	Negative Control			
	STANDARD 1	Standard	20.65	25000000	26176516
	STANDARD 2	Standard	24.30	2500000	2280323
	STANDARD 3	Standard	27.53	250000	261765
	AC tap	Unknown			

Fig 4:Real time PCR of Aqueous aspirate for *M. tuberculosis* DNA-Negative after 2 months



No.	Colour	Name	Type	Ct	Given Conc (IU/ml)
1		NC	Negative Control		
2		STANDARD 1	Standard	19.29	25000000
3		STANDARD 2	Standard	22.95	2500000
4		STANDARD 3	Standard	26.50	250000
5		STANDARD 4	Standard	29.49	25000
6		STANDARD 5	Standard	34.24	2500

Fig 5: Real time PCR of Aqueous aspirate on control sample

DISCUSSION

Tuberculosis is one of the causes of serpiginous choroiditis but serpiginous choroiditis due to autoimmune aetiology exists as an independent entity with distinct clinical characteristics. RT-PCR can detect active replicating TB bacilli and MTB DNA and a negative anterior chamber tap result can indicate the response to treatment. Patients particularly in *tuberculosis* endemic areas may have fundus changes that resemble serpiginous choroiditis but show evidence of *M. tuberculosisDNA* in the

aqueous humor. A substantial contribution may be from an underlying infection and the likelihood of this being tuberculosis is high.

Serpiginous choroiditis in the Asian Indian population is seen in younger individuals with three distinct presentations that can resemble tubercular choroiditis.[5]The ocular morbidity in Indian patients with active tuberculosis was reported as 1.39% and the most common ocular finding was bilateral healed focal choroiditis (50%).[6] Patients with evidence of active or latent tuberculosispresent with serpiginous like clinical features that can resemble the autoimmune type. This has been described as tubercular serpiginous like choroiditis.[7, 8]. An atypical picture of serpiginous choroiditis has been reported in association with toxoplasmosis[9] and herpes virus[10]suggesting that aetiology of infection is indeed possible. The advantage of the ease of anterior chamber paracentesis[11] to diagnose posterior segment inflammation can be of immense help in establishing the identity of tubercular posterior uveitis. Utility of *QuantiFERON TB Gold test* positivity in serpiginous choroiditis indicating latent tuberculosis has been reported.[12.]Apart from ESR, tuberculin skin test and *QuantiFERON TB Gold test* a polymerase chain reaction on anterior chamber aspirate to identify the genome is recommended.[13] We have earlier reported mycobacterium tuberculosis DNA in aqueous aspirates from a case of disseminated tuberculosis.[14] RT-PCR is a reliable investigation in infectious posterior uveitis.[15] Even in situations where all other systemic and ocular investigations were negative RT-PCR was positive thus providing a diagnosis. We feel that apart from providing evidence of MTB DNA it detects the absence of the bacilli in a few months and thus helps to assess response to treatment at a very early stage.

CONCLUSION

The utility of RT-PCR to detect *M. tuberculosis* in serpiginous choroiditis has never been reported and our results provide evidence that RT-PCR, on the aqueous humor can be applied to establish the diagnosis with certainty. It has the potential to significantly improve detection by virtue of its exquisite specificity and follow up for a longer period of time will help to evaluate progress and the recurrence pattern. In view of the ease of performing anterior chamber tap, the ability of RT-PCR to identify the presence of *M. tuberculosis* DNA and the potential of this test to detect the response to treatment, we recommend the use of this procedure to determine whether or not tuberculosis is the aetiology and to provide quantitative assessment of the bacterial load in the eye.

The presence of confirmatory *M. tuberculosis* DNA found by RT-PCR in only one case of 29 patient's points out of the controversy of associating serpiginous choroiditis with tuberculosis. Our study indicates that this association could be a chance association (in an endemic country as India) or if present, a very rare association. Vitreous aspirate analysis by RT-PCR may provide more conclusive evidence by detecting *M. tuberculosis* DNA in patients with serpiginous choroiditis.

Conflict of interest: None

REFERENCES

1. Abrez H, Biswas J, Sudharshan S. Clinical profile, treatment, and visual outcome of serpiginous choroiditis. Ocul Immunol Inflamm. 2007; 15(4):325-35
2. Sahu DK, Rawoof A, Sujatha B Macular serpiginous choroiditis. Indian J Ophthalmol. 2002;50(3):189-96
3. Erkkila H, Laatikainen L, Jokinen E: Immunological studies on serpiginous choroiditis. Graefes Arch Clin Exp Ophthalmol 1982;219:131–34
4. Gupta V, Gupta A, Rao NA. Intraocular Tuberculosis—An Update. Surv Ophthalmol 2007;52(6):561-87
5. Gupta V, Anitha A, Gupta A, Bambery P, Narang S Clinical characteristics of serpiginous choroidopathy in North India. American Journal of Ophthalmology.2002:134(1);47-56
6. Biswas J, Badrinath SS. Ocular morbidity in patients with active systemic tuberculosis. Int Ophthalmol. 1995-1996; 19(5):293-98
7. Bansal R, Gupta A, Gupta V, Dogra MR, Sharma A, Bambery P. Tubercular serpiginous-like choroiditis presenting as multifocal serpiginoid choroiditis.Ophthalmology.2012 ;119(11):2334-42
8. Gupta V, Gupta A, Arora S, Bambery P, Dogra MR, Agarwal A. Presumed tubercular serpiginous like choroiditis. Clinical

presentations and management. Ophthalmology. 2003; 110: 1744–49
9. MahendradasP,KamathG, MahalakshmiB, Shetty KB. Serpiginous choroiditis-like picture due to ocular toxoplasmosis. Ocul Immunol Inflamm. 2007;15(2):127-30
10. Priya K, Madhavan HN, Reiser BJ, Biswas J, Saptagirish R, Narayana KM, et al. Association of herpes viruses in the aqueous humor of patients with serpiginous choroiditis: A polymerase chain reaction-based study. Ocul Immunol Inflamm. 2002; 10:253–61
11. Rothova A, de Boer JH, Ten Dam-van Loon NH, Postma G, de Visser L, Zuurveen SJ, Schuller M, Weersink AJ, van Loon AM, de Groot-Mijnes JD. Usefulness of aqueous humor analysis for the diagnosis of posterior uveitis. Ophthalmology. 2008; 115:306-11
12. Sudharshan S,Ganesh SK, Balu G, Mahalakshmi B, Therese LK, Madhavan HN, Biswas J. Utility of QuantiFERON®-TB Gold test in diagnosis and management of suspected tubercular uveitis in India. IntOphthalmol. 2012; 32:217-23
13. Scheepers MA, Lecuona KA, Rogers G, Bunce C, Corcoran C, Michaelides M. The Value of Routine Polymerase Chain Reaction Analysis of Intraocular Fluid Specimens in the Diagnosis of Infectious Posterior Uveitis. Scientific world journal.2013; 545149. doi: 10.1155/2013/545149
14. Biswas J, Shome D. Choroidal tubercles in disseminated tuberculosis diagnosed by the polymerase chain reaction of aqueous humor Ocular Immunology Inflammation 2002;10(4): 293-98
15. Santos FF, Commodaro AG, Souza AV, Pinho JR, Sitnik R, Garcia C, Ribeiro AL, etal., Real-time PCR in infectious uveitis as an alternative diagnosis. 2011;74:258-61