Comparison of Polymerase Chain Reaction Results with Treatment Response in the Diagnosis of Infectious Uveitis

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ABSTRACT

Introduction: Analysis of polymerase chain reaction (PCR) results v/s clinical response in infectious uveitis will unravel the diagnostic enigma. Study aimed to retrospectively compare the PCR outcome with final treatment response outcome in infectious uveitis.

Material and methods: Retrospectively 35 patients who underwent UNIPLEX and NESTED PCR during 2013-15, from aqueous/vitreous tap were analyzed. Follow up ranged from 3-19 months. With strong clinical suspicion, treatment was initiated and the clinical responses were compared to validate PCR results.

Results: Among 35 cases, 22 (62%) were presumed ocular tuberculosis, 10(28%) were viral uveitis, 2 eyes each as Propionobacterium acne and 1 eye as ocular toxoplasmosis. Estimated sensitivity, specificity, positive and negative predictive value for tuberculosis subgroup were 42%, 80%, 71% and 53% respectively and for viral uveitis it was 33%, 100%, 100% and 15% respectively.

Conclusion: Isolating organism in infectious uveitis is challenging and clinician depends on therapeutic trial. In clinical dilemma positive PCR can be a better tool to confirm the disease but the clinical judgment prevails over negative PCR results.

Keywords: Infectious Uveitis, Polymerase chain reaction, Treatment response

INTRODUCTION

Polymerase chain reaction (PCR) is a novel diagnostic technique used in various sub-specialties. Role of PCR in ocular disorders has been described in various reviews.[1-10] The gold standard diagnostic investigation in case of infectious uveitis is considered to be isolation of the inciting microorganism. But in cases of posterior ocular infectious uveitis, the paucity of clinical sample, need of early diagnosis and timely intervention abates its usage. In such a scenario, clinical judgment, tailored serological investigations, immune-assays, response to treatment and PCR play an important role. In order to establish the hierarchy of PCR, its sensitivity, specificity, positive predictive value and negative predictive value has been compared with different reference standard.[1,4,6,8] Most of the previous studies have compared PCR with either clinical diagnosis[1,11] or combination of clinical examination with immunoassay,[4] but sparse literature is available on comparison of PCR with treatment response per se. Our study aimed to retrospectively compare the PCR outcome with final treatment response outcome in infectious uveitis.

MATERIAL AND METHODS

The study was conducted in the Uveitis services of a tertiary eye care hospital in South India. Retrospective case records of consecutive patients who were clinically diagnosed as presumed infectious uveitis based on the clinical history, comprehensive ophthalmic examination, systemic and ocular investigations were included for analyses. The study period was from Jan 2013 to Jan 2015. Minimum of three months was taken as cut off for follow up. The provisional clinical diagnosis and differentials were considered taking into account both the typical and atypical presentation of infectious uveitis. Defining all the criteria is not feasible considering the limitation of article. All the cases underwent both UNIPLEX and NESTED PCR (institutional medical research foundation) during 2013-15, from aqueous or vitreous tap were analyzed. NESTED PCR was used to increase the specificity of the amplification process. Due informed consent was obtained from all the patients before collecting aqueous or vitreous aspirate for PCR. The study was approved by the Institutional Review Board and has been carried out in accordance with Declaration of Helsinki. The data regarding clinical presentation, provisional diagnosis, treatment and response outcome were extracted from the case records. Empirical treatment was started on the basis of clinical examination till the results of PCR were obtained following which the treatment was modified if needed. Treatment response was taken as reference standard for estimating PCR indices (sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]).

STATISTICAL ANALYSIS

Microsoft excel 2010 (Microsoft Office Standard 2010) was used for data entry and further calculations were done with the help of descriptive statistics.

RESULTS

Thirty nine eyes of thirty nine patients were found to be eligible for the study. Four eyes were excluded due to inadequate data. The follow up ranged from 3-19 months. Out of 35 eyes, 22 eyes were diagnosed clinically as presumed ocular tuberculosis, 10 eyes as viral uveitis and 2 eyes each as Propionobacterium acne and 1 eye as ocular toxoplasmosis. The indices obtained after comparing the PCR results and treatment response are listed in Table 1 and 2. The different primers used for the organisms were depicted in table-3.

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When analyzing the result for tuberculosis, it was found that among 22 eyes both the PCR results and the treatment response were simultaneously positive in 5 eyes (true positive). The concurrence of PCR negativity and negative response to treatment were observed in 8 eyes (true negative). The estimated sensitivity for tuberculosis is 42%, specificity 80%, positive predictive value as 71% and negative predictive value as 53%.

While interpreting the results for viruses, it was found that among 10 patients, concordance of positivity of PCR report and treatment response was seen in 3 patients (true positivity). The concurrence of PCR negativity and negative treatment response

Organism	Sample number	True positive	False positive	True negative	False negative
TB	22	5	2	8	7
Viral	10	3	0	1	6
Overall	35*	10	2	9	14
*Includes 3 eyes, 2 of Propionibacterium acne uveitis and 1 of toxoplasmosis uveitis					

Table-1: Final outcome after co-relating the PCR results and treatment response

Estimated indices	Sensitivity (%)	Specificity (%)	PPV*(%)	NPV** (%)
TB	42	80	71	53
Viral	33	100	100	15
Overall[#]	42	82	83	39
* PPV – Positive Predictive value; **NPV – Negative Predictive value; # Includes 3 eyes, 2 of Propionobacterium acne uveitis and 1 of toxoplasmosis uveitis				

Table-2: Measure of PCR validity compared to treatment response as the reference standard

S. No	Organisms	Genes	PCR	Primer sequence	PCR Product length	References
1.	Mycobacterium tuberculosis	MPB64	Uniplex	FP: 5' TCCGCTGCCAGCCGTCTTCC 3' RP:5' GTCCTCGCGAGTCTAGGCCA 3'	240 bp	KL Therase et al., 2005
			Nested	FP: 5' ATTGTGCAAGGTGAACTGAG 3' RP: 5' AGCATCGAGTCGATCGCGA 3'	200bp	
2.	Propionibacterium acnes	16 S rDNA	Uniplex	FP: 5' AAGGCCCTGCTTTTGTGG 3' RP: 5' ACTCACGCTTCGTCACAG 3'	387bp	KL Therase et al., 1998
			Nested	FP: I round F-Primer RP: 5' TCCATCCGCAACCGCCGAA 3'	160bp	
3.	Toxoplasma gondii	B1 Gene	Uniplex	FP:5' TGC ATA GGT GTC AGT CAC TG 3' RP: 5' GGC GAC CAA TCT GCG AAT ACA CC 3'	193bp	Colin D Jones et al., 2004
			Nested	FP: 5' TGC ATA GGT TGC AGT CAC TG 3' RP: 5' GGC GAC CAA TCT GCG AAT ACA CC 3'	96bp	
4.	Varicella Zoster Virus	Immediate early gene 63	Uniplex	FP: 5'GTT TTG TAC TCC GGG TTG 3' RP: 5' TTA CAT CCG ATG GCG TAG 5'	387bp	Priya K,et al.,2000
			Nested	FP: 5'GCG CGT TGA GGA CAT CAA CCG TGT T 3' RP: 5' CAT CGT CGC TAT CGT CTT CAC CAC 3'	326bp	
5.	Herpes Simplex Virus	Glycoprotien D gene		FP: 5' CGA AGA CGT CCG GAA ACA AC 3' RP: 5' CGG TGC TCC AGG ATA AA 3'	297bp	Madhavan HN, et al.,1999
				FP: 5' CGA AGA CGT CCG GAA ACA AC 3' RP: 5' TCT CCG TCC AGT CGT TTA TCT TC 3'	272bp	
6.	Cytomegalo Virus	MTR Gene		FP: 5' CTG TGG GTC ATG GTC TCT TC 3' RP: 5' CCC GAC ACG CGC AAA AGA AA 3'	234bp	Priya K,et al 2002
				FP: 5' TCT CTG GTC CTG ATC GTC TT 3' RP: 5' GTG ACC TAC CAA CGT AGG TT 3'	168bp	

Table 3: Primers used in the analysis of polymerase chain reactions in the different sub-groups:

Parameters	Sugita et al (2013)	Scheepers et al (2013)	Harper et al (2009)	Our study (2015)
Study type	Prospective	Retrospective	Retrospective	Retrospective
Sample size	500	159	133	29
Type of PCR	Multiplex and broad range RT	Nested	Real time	Uniplex and Nested
Agent tested*	HHV 1-8, Bacteria and fungi	CMV, HSV 1 and 2, VZV, TG, MTB	HSV 1 and 2, CMV, VZV, Toxo	CMV, HSV 1 and 2, TG, MTB, P. acne
Follow up	Not mentioned	1 week – 5 years	6 months	3 – 19 months
Sensitivity (%)	91.3	84	80.9	42
Specificity (%)	98.8	99	97.4	82
PPV (%)	98.6	97	98.7	83
NPV (%)	92.4	95	67.9	39
PCR v/s	Examination Clinical findings Treatment response	Final diagnosis (clinical exam + investigations + treatment response)	Final diagnosis	Treatment response

*HHV – Human Herpes virus, HSV- Herpes simplex virus, CMV- Cytomegalovirus, VZV- Varicella Zoster Virus, TG- Toxoplasmosis gondii, MTB – Mycobacterium Tuberculosis, P. acne – Propionobacterium acne

Table-4: Shows a comparative analysis of our study with the few previous available literature

was seen in only 1 patient (true negativity). This results in a sensitivity and specificity of 33% and 100% with the positive and negative predictive values of 100% and 15% respectively.

DISCUSSION

The gold standard diagnostic modality for any ocular infectious uveitis is isolation of micro-organism but it is not always feasible due to the paucity of sample and time consuming isolation technique. Establishing an etiological diagnosis in infectious uveitis based on clinical grounds has limitations as same micro-organism could have different clinical presentations and different organisms might have similar presenting features. In such a scenario PCR of intraocular fluids can aid in the diagnosis.

The PCR is an expensive diagnostic tool and is not readily available in poor resource settings. PCR test results should be valid and reliable for it to be useful to the clinician in infectious uveitis. To establish its validity, it is important to compare it with the reference standard like clinical presentation and treatment response. In our study we compared it with treatment response as the reference gold standard for diagnosing infectious uveitis. The reason we took only treatment response was to be able to imply the PCR results in actual clinical scenario, where due to poor resources most of the clinician would not have the liberty to conduct a battery of investigations. In dilemmatic cases it is important for an ophthalmologist to decide on whether which investigation would aid the most for diagnostic purpose and hence modified the empirical treatment, if needed. Therefore establishing the validity of PCR results with the treatment response would bypass the need of an array of investigation to arrive at a confirmative diagnosis.

Recently Scheepers et al. who discussed on the value of PCR in infectious posterior uveitis have documented a high sensitivity, specificity, positive predicted value (PPV) and negative predicted value (NPV) of 84%, 99%, 97% and 95% respectively. The data was in concordance with the previous similar studies.[1,8,9]

In our study (Table 4) the overall sensitivity and NPV were 42% and 39% which is almost half as compared to previous studies[1,8,9] suggesting a higher false negative rate in our study. This could be the result of sequence polymorphism, inadequate sample quantity and problem with cold chain maintenance during transportation of aspirate. Lower sensitivity and NPV of PCR test should be taken into consideration while extrapolating the negative PCR results in establishing the final diagnosis. The estimated sensitivity and NPV in presumed tuberculosis (42% and 53% respectively) was relatively more than viral uveitis (33% and 15% respectively). Hence, negative PCR in viral uveitis have to be more cautiously interpreted and the treatment response will always supervene in establishing the final diagnosis.

In contrary, the high specificity and positive predictive value (82% and 83% respectively) in our study supports the fact that PCR is a very reliable diagnostic tool if the results are positive. This finding has been similar to the previous studies[1,8,9] and probably due to widespread use of NESTED PCR. A 100% specificity and PPV in viral uveitis might confirm presence of inciting agent in all positive PCR cases. Thus, in conjunction with clinical response, positive PCR results are given a higher weightage. In case of tuberculosis the higher specificity and PPV (80% and 71%) points to a similar extrapolation.

CONCLUSION

As per our study results a positive PCR in infectious uveitis supports the treatment response. Thus it can be considered as a reliable tool for diagnosis and starting treatment especially in viral etiology. But, overall negative PCR results have to be more cautiously interpreted and the treatment response will always supervene in establishing the final diagnosis.

Limitation

This is a retrospective study with its own limitations and the small sample size restricts widespread implication of such results. Hence, further prospective studies of adequate representative sample size are needed to prove its applications.

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