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Polymerase chain reaction in uveitis

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Abstract
Polymerase chain reaction (PCR) has opened new vistas in the diagnosis of various diseases. It involves enzymatic amplification of nucleic acid sequences within a short period. Polymerase chain reaction has greater role in diagnostic pathology, especially in microbiology, genetics, and oncology. Identification of infectious microorganisms, ruling out infection as the cause in the eyes of patients with uveitis to ensure prompt diagnosis and appropriate therapy in ophthalmology is of paramount importance. Infectious uveitis in India needs early diagnosis of the responsible pathogen and administration of appropriate treatment can help reduce visual impairment. Polymerase chain reaction analysis is a highly sensitive and specific method to detect microbial DNA in ocular samples from immunocompetent and immunocompromised patients with uveitis. In this review article we explore the role of PCR in uveitis citing appropriate examples.

Keywords: Polymerase chain reaction (PCR); ocular /ophthalmic; uveitis; Qualitative PCR; Quantitative PCR;

Introduction

Polymerase chain reaction (PCR) is a revolutionary technology which has opened a new era in the diagnosis of various diseases and involves enzymatic amplification of nucleic acid sequences within a short period.

It was developed by Kary Mullis in 1985 and he was awarded the Nobel Prize in Chemistry along with Michael Smith in 1993 [1]. Polymerase chain reaction uses the property of the enzyme DNA polymerase to synthesize new complimentary DNA strand with the help of a primer. Hence a specific region of template sequence can be amplified to billions of copies as per requirement [2].

Polymerase chain reaction has found numerous applications in diagnostic pathology, especially in the fields of microbiology, genetics, and oncology. In Ophthalmology it helped us to identify infectious microorganisms, and also rule out infection as the cause in the eyes of patients with uveitis to ensure prompt diagnosis and appropriate therapy. Application of PCR in the detection and diagnosis of infectious uveitis caused by various viruses, bacteria, and fungi in ocular tissues and/or fluids has been described [3, 9]. The prevalence of infectious uveitis in India is reported to be as high as 31% [10]. Early diagnosis of the responsible pathogen and the subsequent prompt administration of appropriate treatment can help to reduce visual impairment. Polymerase chain reaction analysis is a highly sensitive and specific method to detect microbial DNA in ocular samples from immunocompetent and immunocompromised patients with uveitis [11, 12]. A sensitivity of 81% and specificity of 97% was noted by Harper *et al* for PCR in posterior uveitis [13].

Interpretation of PCR along with a detailed history and clinical examination are useful tools to ensure accurate diagnosis. Real-time PCR (RT-PCR), has become extremely useful in clinical diagnostics and research due to its ability for generating Quantitative results in a faster and more accurate fashion than conventional PCR, which only displays the qualitative results [14]. The aim of the present literature review is to explore the clinical usefulness and potential of both conventional PCR and Real-Time PCR assays in uveitis and addressing its main uses and advances.

Materials and Methods

A comprehensive literature search was conducted on MEDLINE, PubMed, and Google Scholar databases in November 2019. Search temporal limits enclosed articles printed from 1980 to November 2019 with the aim of providing the foremost recent proof. Studies were queried using the subsequent keywords in varied combinations: *"PCR," "ocular," "uveitis," "Polymerase chain reaction", and "ophthalmic uses*."(Articles enclosed for the review provided an outline of importance of PCR for diagnosis of uveitis, its indications, methods, outcome and significance. English language articles were included for this review. Articles, like preliminary reports and abstracts, were excluded as they failed to have descriptive data.

1. Principle of PCR Technology

Polymerase Chain Reaction also called as "Molecular Phototyping" is a technique used to amplify small and targeted segments of DNA to produce millions of copies of a specific gene fragment. Figure 1 shows the instruments used in the process.

The three main steps of PCR include [15]

a. De-naturation
b. Annealing
c. Extension

Fig 1: DNA Extraction from Samples.

A single PCR process can be completed in 30-35 cycles completed in 2 hours' time to amplify to produce usable amount of DNA fragments.[11]Different types of PCR process with slight modifications can be used to produce better and faster results. The following are the essential components of PCR

- Thermocyclers -They heat and cool the reaction tubes to achieve the optimum temperature.
- Target DNA and nucleotides -They consist of the segments which are to be amplified and represent a small part of a large and complex mixture of a specific DNA of a genome.[16]
- Two primers -They are short segments of DNA made of less than 50 nucleotides which limit the DNA sequence to be replicated.
- Taq polymerase - is derived from *Thermus aquaticus* and are the standard reagents for PCR reaction. *Thermus aquaticus* is a species of bacteria that can tolerate high temperatures, one of several thermophilic bacteria that belong to the Deinococcus.
- Buffers -It includes mainly magnesium chloride which supplies magnesium divalent cations useful as a co- factor. The other PCR buffers used are DMSO (Dimethyl sulfoxide), PEG (Polyethylene glycol), 6000, Glycerol formamide etc.
- DNTP'S -these deoxy nucleotide triphosphate supplies the nucleotides to Taq polymerase enzyme to synthesize a new strand of DNA.
- Denaturing /melting

Denaturation

The first step involves heating the DNA molecule to a temperature of 94-96 ℃ for 10 -20 minutes causing the 2 complementary strands to split using Taq polymerase enzyme.

Annealing (hybridization)

It includes attaching the primer to the split single strand DNA after lowering the temperature. This process takes place in about 1-2 minutes and each strand of DNA molecule becomes annealed with a primer complementary to either and of target sequence.

Extension Now the DNA polymerase extends the chain by filling nucleotides in the 3' 5' direction away from each of the primer at a temperature of 72 C for 5-15min.The amplified product of PCR process called as amplicon.

Qualitative PCR techniques

When PCR techniques are used for detecting a specific DNA segment, it is called as qualitative PCR method. PCR techniques are used in the identification of genes of bacteria and virus.

Quantitative PCR techniques

It is also referred to real time PCR. It quantifies the DNA amount present in the sample.

Real-Time PCR is a highly sensitive method which allows the precise quantification of these nucleic acids with greater reproducibility.

Fluorescent composites like *SYBR®Green (*asymmetrical cyanine dye) *and* Taq Manare used to bind to double stranded DNA in RT-PCR and the fluorescent signals are captured with an optical system. These signals are analyzed using computer software and the fluorescence generated is directly proportion with the amount of PCR products.[17]

2. Role in Uveitis

Infectious and non-infectious uveitis often share similar clinical picture at presentation, hence obtaining an early definitive laboratory-proven diagnosis is very vital in start in commencing effective treatment and preventing complications.

A. Application in Viral uveitis

PCR has been useful in qualitative and quantitative diagnosis of viral nucleic acids with complete viral characterization, determining the subtype, genotype, variation, mutation and standards of genotypic resistance of these viruses [18].

The implementation of molecular strategies has resulted in rapid diagnosis of many viruses and also in monitoring of antiviral treatment, particularly Human Immunodeficiency Virus type 1, Hepatitis B Virus, Human Cytomegalovirus, Dengue virus, Chikungunya virus [19, 25].

Real-Time PCR is highly sensitive and specific for detection and quantitation of dengue viruses and can differentiate it from other clinically indistinguishable infectious diseases like malaria, Chikungunya, rickettsia and leptospira [22, 23] qPCR using probes can be a powerful tool to detect CHIKV RNA (chikungunya viral genetic material) during the acute phase of the disease [24]. Additionally it provides amplification assays on nearly all human viruses [25].

The introduction of molecular biology with automatic extraction and detection have power assisted in quicker clinical diagnosis and reduced the use of infective agent culture techniques [26].

Real-time PCR is also an attractive alternative to conventional PCR for the study of viral load because of its low inter-assay and intra-assay variability [27]. Viral load is also a useful indicator of the extent of active infection, virus–host interactions and the response to antiviral therapy, all of which can play a role in the treatment regimen selected [28].

The ability to exclude viral infections can help avoid unnecessary therapies, such as powerful antibiotics and antiviral medicines, as well as reduce costs incurred on the part of patients.

The majority of the viruses associated with eye diseases are related to the herpes virus group. This family includes herpes simplex virus (HSV) types 1 and 2, varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), and human herpes virus types 6, 7, and 8. The main viruses responsible for ocular inflammation are HSV type 1, VZV, and CMV. EBV and HSV type 2 have also been detected in ocular fluids of patients with anterior uveitis, though less frequently [29].

Ocular viral manifestations include keratitis, uveitis and acute retinal necrosis. In a uveitic presentation associated with characteristic keratitis the diagnosis is straightforward, but in recurrent iridocyclitis and in the immunocompromised patients the diagnosis is challenging. Cell-free herpes virus DNA has been isolated in the aqueous humour and vitreous fluids of patients with uveitis [30, 33]. Therefore, the last decade has seen several studies concluding that herpes virus PCR-based laboratory investigations are valuable tools in the diagnosis of viral diseases of the eye.

When faced with a clinical situation that suggests a differential diagnosis of HHV1–8, the multiplex PCR [30] assay can provide a rapid and reliable diagnosis, even when only small sample amounts are available for examination in the ocular microbiology laboratory.

The first report of use of PCR in ophthalmology in India was in 1993 for detection of CMV retinitis [31]. PCR sensitivity for CMV retinitis ranges from 91 to 95% [32, 33]. Reliability of the PCR method is similar to in situ DNA hybridization for the detection of CMV however morphologic correlation is provided only by the latter. Figure 2 shows the PCR detection of CMV virus.

PCR on aqueous humour sample allowed identification of the causative agent in acute retinal necrosis from 40% [30] to 86.4% [32].

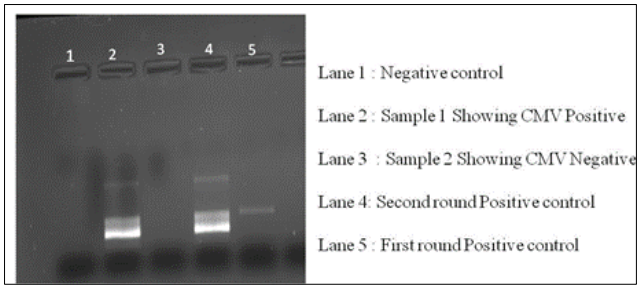

Fig 2: 2 % Agarose gel showing 234 and 168 bp of mtr II region of CMV.

Some of the challenges noted while diagnosing viral uveitis with PCR include dual positivity due to the extreme sensitivity of the test. Certain dormant viruses can be liberated in an inflamed eye resulting in more than one true positive result. Sugita and associates found high levels of EBV DNA by RT-PCR in four eyes with confirmed VZV infection [33]. Figure 3 shows the PCR detection of VZV virus.

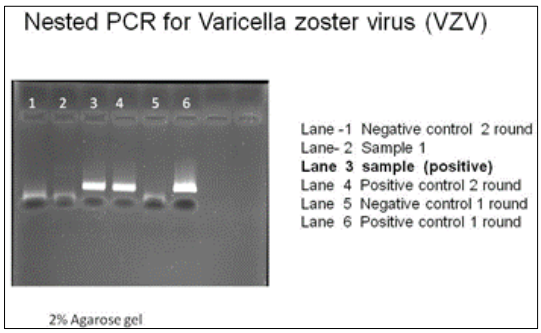

Fig 3: 2 % Agarose gel showing Nested PCR for Varicella zoster virus (VZV)

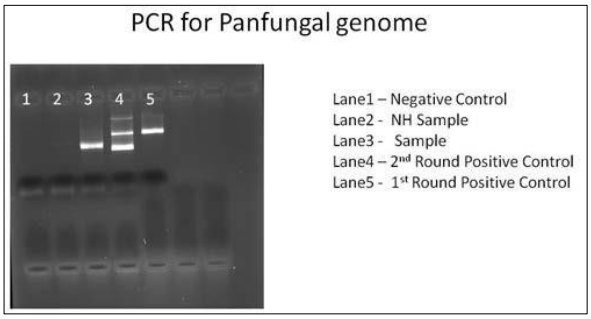

Fig 4: Polymerase chain reaction for panfungal genome.

This can be addressed by using RT-PCR with a cut-off level for numbers of copies to be considered as positive hence reducing the number of false-positive results. RT PCR technique can also be useful in conducting epidemiological studies due to its capacity to quantify nucleic acids in a single reaction and in evaluating the treatment response by quantifying the viral load.

B. Application in Bacterial uveitis

Intraocular infection can occur secondary to bacteria, either introduced into the eye following trauma, surgery or from another source via systemic circulation. Intraocular bacterial endophthalmitis can be devastating for vision. Confirmation of the diagnosis of bacterial endophthalmitis is traditionally based on microbiologic isolation of organisms, but many cultures are found to be negative (21%–63%) [34, 35]. The reasons for these negative results can be sequestration of bacteria on solid surfaces, prior use of antibiotics, and the fastidious nature of some of the organisms that cause intraocular infection.[36] Bacterial DNA was successfully identified using PCR in 100% of samples from patients with typical clinical signs of bacterial endophthalmitis and vitreous was shown to be the sample of choice for both PCR and microbiologic analysis [36].

In a study by Joseph *et al*, in 64 patients who developed post cataract surgery endophthalmitis, PCR allowed the detection of bacterial DNA in 66% of patients, compared to 34% with traditional culture and sensitivity. Only one patient had a positive result by culture (Nocardia species) but negative result by PCR [37].

False positivity is attributed to the presence of contaminants, hence the collection protocol is stricter for PCR analysis.

Tuberculosis is implicated in intraocular inflammation either causing direct infection of intraocular tissues or resulting in immune-mediated inflammation. PCR not only helps in prompt diagnosis of MTB (Mycobacterium Tuberculosis) but also helps in quantifying the mycobacterial load using qPCR amplification technique. Sclera-corneal scrapping, aqueous and vitreous humor, subretinal fluid, ERM and even tissue specimens have been used for PCR testing of MTB DNA, with promising results. GeneXpert™ assay is a another useful technique for prompt detection of MTB and drug resistance to Isoniazid and Rifampicin [38, 39]. The Xpert MTB/RIF assay uses molecular beacon technology to detect DNA sequences amplified in a heminested real-time-PCR assay [40].

Another technology Xcyton™ involves isolation of the genetic material of the causative agent from the given specimen and simultaneous amplification of the "Syndrome Specific Signature

genes" of all the probable causative agents, followed by "Syndrome Specific Hybridization". It has high sensitivity and specificity, and simultaneous detection of multiple pathogens allows for early diagnosis of the infection and initiation of therapy [41]. A case report showed that Multiplex PCR-based DNA chip technology (Xcyton) can be used to investigate a previously unsuspected etiology in Intermediate uveitis particularly in recurrent cases.[42] Xcyton analysis can also be used for the diagnosis of polymicrobial endophthalmitis and in culture negative endophthalmitis [43].

C. Application in parasitic and fungal uveitis

Parasitological diagnostics can be assisted by molecular methods. Many parasites are not cultivable in laboratory and diagnosis principally relies on serology and relatively less sensitive microscopy.

Toxoplasma gondii is a protozoan parasite that can affect many species of warm-blooded animals, including human. *T gondii* is one of the most important parasites responsible for uveitis and retinochoroiditis. Ocular toxoplasmosis is the most common type of infectious posterior uveitis worldwide [44] The B1 gene with conserved sequences in almost all *T. gondii* strains, have been used for the detection of parasite in various clinical samples including ocular fluids, and reported to have acceptable sensitivity and specificity [45]

Montoya *et al.* [46] were able to detect Toxoplasma DNA in nearly 80% of patients with suspected ocular toxoplasmosis and positive serum IgG titers.

The PCR of vitreous samples, comparing to the aqueous humor, showed a higher sensitivity in diagnosis of ocular toxoplasmosis, (95.7% vs 81.5%) [47].

Positive fungal results usually take longer than a week due to the slow growing nature of these organisms. Early diagnosis and rapid intervention is a critical element for an effective treatment of ocular infections. This has led to the development of culture-independent diagnostic tests such as PCR. For detection of fungal pathogens, multicopy gene targets have been evaluated for increasing the sensitivity and universal fungal PCR primers have been developed for broadening the range of detectable fungi [48]. Figure shows PCR of a panfungal genome.

The use of rRNA genes for identification of fungal species is based on the detection of conserved sequences in the rDNA genes [49]

Another rapid advance in diagnosis of uveitis is Metagenomic deep sequencing which can identify fungi, parasites, and DNA and RNA viruses in minute volumes of intraocular fluid samples. It can detect rare pathogens in eye which can be a valuable public health tool to survey for re-emerging and emerging infectious diseases in immune privileged body sites [50, 51].

PCR techniques also play an integral role in targeted Next generation sequencing, allowing for the generation of multiple NGS libraries and the sequencing of multiple targeted regions simultaneously [52].

As genetically modified (GM) technology develops and genetically modified organisms (GMOs) become more available, combining the advantages of multiplex detection and droplet digital polymerase chain reaction (ddPCR), a single universal primer-multiplex-ddPCR (SUP-M-ddPCR) strategy was proposed for accurate broad-spectrum screening and quantification [53].

A recent multicentre study showed Strip PCR is recommended for rapid diagnosis of infectious uveitis as its results are equivalent to that of conventional qPCR [54].

D. Application in non-infectious uveitis

The most common application of PCR in non-infectious uveitis is HLA typing. Shino *et al.* reported complete association of the HLA-DRB104 and DQB104 alleles with Vogt-Koyanagi-Harada (VKH) disease using PCR technique [55].

Intraocular lymphoma is an important masquerade of intermediate uveitis. In most cases, intraocular lymphoma involves the vitreous and the choroid and is a non-Hodgkins CD20+ B cell lymphoma, which is part of the spectrum of central nervous system (CNS) lymphoma. Approximately, 25% of patients with primary CNS lymphoma of this type develop intraocular involvement. Conversely, patients presenting with intraocular lymphoma have a high risk of developing CNS pathology, with over 50% developing disease [56]

Utilization of PCR has become a practical tool for the detection of IgH gene rearrangements and provides a helpful adjunct for the diagnosis of B-cell lymphoma in the eye [57].

The allele - specific PCR melting assay for Human leukocyte antigen HLA - B27 genotyping is easy to perform and has better sensitivity and specificity than antigen assays [58].

Drawbacks of PCR technology

Organism from systemic circulation gets into ocular fluid without causing the disease. These dormant organisms are detected on PCR in disease free eyes. A study showed MTB genome in the subretinal fluid containing RPE cells from individuals with latent TB, who did not have any evidence of intraocular TB or manifest systemic TB disease [59]. PCR based Next generation sequencing may detect irrelevant oraganisms [60]. The chance of laboratory contamination is a major risk. PCR can even detect microorganisms shed from the laboratory personals. It can detect DNA of dead colonising normal flora of the conjunctiva. However these problems can be to some extent overcome with the advent of newer techniques such as real time PCR. High specificity can give rise to false-negative results, if the target DNA location of the pathogen is pleomorphic [61]. PCR cannot detect the organism for which primers have not been provided. So a narrow and well defined differential diagnosis is required for PCR to be effectively useful [62].

Discussion

Our review with the key words“PCR,” “ocular,” “uveitis,” and “ophthalmic uses” yielded 21 (15 infectious and 6 non-infectious) articles. A total of 295 specimen in non-infectious uveitis and 1312 specimen in infectious uveitis were included. Table 1 and 2 summarizes the details of PCR in infectious and non-infectious uveitis in the articles that were included for the purpose of this review. There were 4 case reports and 17 case series. The following methods of PCR was used – Light cycler PCR, Nested, Semi nested and Multiplex PCR, out of which nested PCR was the most common method used in above case series.

PCR based techniques were useful in confirming bacterial endophthalmitis in culture negative eyes and aid in early

diagnosis of tuberculosis from small samples.[5,6] It can be a rapid tool for detecting Fungi in ocular samples and can help in early diagnosis and monitoring of antifungal therapy [9].

It is superior to other methods for quantification of HBV DNA in sensitivity, specificity, simplicity, and reproducibility [27].

There were 6 case series on viral uveitis which showed that PCR is useful as a screening tool for screening viral infection [13, 25, 27, 31, 32, 33]. B1 gene detection by nested PCR was known to be useful in the diagnosis of Ocular toxoplasmosis [47]. Rarely VZV reactivation which was detected by multiplex PCR, can present as frosted branch angiitis and hence should be a differential diagnosis for retinal perivasculitis [55]. Crucial role of Ser at position 57 of DRB1 and/or Glu at position 70 and Asp at position 71 of DQB1 was noted by PCR-RFLP in VKH disease.[63]Combination of microdissection and PCR helps in the diagnosis of masquerades even in small sample volume [64]. Also a significant increase of the HLA-B*5101 allele was found using the PCR- sequencing based typing in Behcets disease [66]. Another series on molecular analysis of resolving immune responses studied using Radiometric and RT PCR analysis in various non-infectious uveitis showed a predominant T cell mediated inflammatory response [67]. The rubella virus etiology in Fuch's Heterochromic Iridocyclitis studied using nested PCR was found to be more associated in younger patients.[68] This etiology highlights the altered pathomechanisms in FHIC and the poor response to corticosteroid therapy in such patients.

Table 1: PCR in Infectious Uveitis

Articles	Design	Number of Cases	Infectious Agent	Type of PCR Used	Results	Comments
Faber *et al* [5]	Case series	30 eyes with recurrent uveitis and 16 controls	Leptospira	PCR	Twenty-one (70%) of the horses with uveitis (*n* 5 30) had leptospiral DNA in the aqueous humor detectable with PCR	PCR is a more reliable tool for detecting the presence of leptospires in equine recurrent uveitis
Kotake *et al* [6]	Case reports	2 cases	Tuberculosis	PCR		Early diagnosis of tuberculosis from small samples and early medical intervention in cases of M. *tuberculosis* infection can contribute not only to therapeutic improvement of affected individuals, but also to prevention of the disease and protection of medical staff.
Alexandrakis *et al* [9]	Case report		Fusarium	PCR	Short fragment of *Fusarium* DNA Was amplified from 3-year-old ocular pathology specimens to detect *Fusarium* DNA from the paraffin sections, as well as from the formalin-fixed retinal, choroidal, and scleral tissues.	PCR is sensitive, specific, and rapid method of diagnosing ocular *Fusarium* infections, as well as *Fusarium* infections at other sites.
Harper *et al* [13]	Case series	133 patients with suspected infectious uveitis	HSV 1 and 2, VZV, CMV, EBV, Toxoplasma gondi, HTLV	….	Sensitivity and specificity of 80.9% and 97.4%, respectively	Cases sampled within one week of presentation were more likely to have positive PCR results than those sampled later. The predictive value of positive and negative tests was 98.7% and 67.9%, respectively
Mark J Espy *et al* [25]	Case series	200 specimens (number of genital specimens, 160; number of dermal specimens, 38; number of ocular specimens, 2)	HSV 1 and 2	Light Cycler PCR	Sensitivity 78% Specificity-100%	Increased sensitivity of the Light Cycler PCR compared to cell culture methods was noted
Abe *et al* [27]	Case series	46 patients and 23 healthy volunteers	Hepatitis B	real-time detection PCR	detected HBV DNA in 100% of chronic hepatitis B patients tested and never detected HBV DNA in healthy volunteers who were negative for HBV markers	RTD-PCR assay for HBV DNA is superior to other methods for quantitation of HBV DNA in sensitivity, specificity, simplicity, and reproducibility
Biswas *et al* [31]	Case series	16 eyes	CMV			PCR may be more sensitive than in situ DNA hybridization in the detection of human CMV,
T H C Tran *et al* [32]	Case series	19 eyes with necrotising Herpetic retinitis	HSV-1, 2, VZV, CMV	Multiplex PCR	Sensitivity-86.4%	Reliable identification of the pathogenic agent was possible by PCR analysis

S. Sugita *et al* [33]	Case series	111 eyes with acute retinal necrosis	HHV 1-8	Multiplex PCR and Real time PCR	65 multiplex PCR positive patients 42 real-time PCR positive patients	A qualitative multiplex PCR is useful in the screening of viral infections
Okravi *et al* [34]	Case series	Ninety ocular samples from 78 eyes	Bacterial	Nested PCR	presence of bacterial DNA in 100% of samples from eyes with clinically suspected presence of infection.	PCR-based techniques have great value in the confirmation of the diagnosis of bacterial endophthalmitis especially in culture-negative eyes
Jones *et al* [45]		three *Toxoplasma gondii* genes in aqueous humor.	Toxoplasma gondi	Nested PCR		The B1 PCR protocol appears to be the most sensitive protocol in the detection of *T. gondii* DNA
Farhadi *et al* [47]	Case series	Fifty aqueous or vitreous humor samples were obtained from patients with clinical features of ocular toxoplasmosis	Toxoplasma	Nested PCR	Sensitivity after first round PCR- 58 % After nested PCR- 88%	Nested-PCR protocol using the B1 gene useful complimentary method to clinical diagnosis of ocular toxoplasmosis
Hermann *et al* [48]	Case series	134 fungal and 85 nonfungal isolates, 601 blood samples	Fungi	PCR	Specificity of 98%.	some value in the early diagnosis and monitoring of antifungal therapy
Ferrer *et al* [49]	Case series	11 ocular samples	Fungi	PCR and seminested PCR	sensitivity of the seminested PCR amplification reaction by DNA dilutions was 1 organism per PCR, and the sensitivity by cell dilutions was fewer than 10 organisms per PCR.	this method can be used to differentiate fungi at the species level. Potential as a rapid technique for identifying fungi in ocular samples.
Taher *et al* [55]	Case report		Varicella zoster	Multiplex PCR	Frosted branch angitis can be a rare presentation of VZV reactivation.	VZV should be considered in the differential diagnosis of retinal perivasculitis

Table 2: PCR in Non-Infectious Uveitis

Articles	Design	Number of Cases	Cause of Uveitis	Purpose of Study	Results	Comments
Shindo Y *et al* [63]	Case series	63 eyes	VKH	To study the complete association of the HLA-DRB1*04 and -DQB1*04 allele in VKH using PCR-RFLP	Complete association of the HLA-DRB1*04 and -DQB1*04 alleles with Vogt-Koyanagi-Harada disease (VKH)	Also Indicates that Ser at position 57 of DRB1 and/or Glu at position 70 and Asp at position 71 of DQB1 plays a crucial role in determining the susceptibility to VKH disease.
De Fen Shen *et al* [64]	Case series	5 ocular specimens	Primary CNS Lymphoma	Histologic tissue sections were analyzed using microdissection and polymer- ase chain reaction (PCR) technique for bcl 2 and IgH	Translocation of IgH and bcl-2, the apoptotic "survival" signal and proto-oncogene, could contribute to the pathogenesis of PCNSL.	The combination of microdissection and PCR is a powerful tool for studies of small lesions
Chi-Chao Chan [65]	Case series	57 vitrectomy specimens	Primary CNS Lymphoma	To evaluate immunoglobulin heavy chain (IgH) gene rearrangements, cytokines and chemokines, and infectious agents using RT PCR	IgH rearrangement, elevated vitreal IL-10 and IL-6 levels	B-cell chemokine is likely involved in attracting PIOL cells into the eye. HHV-8, EBV dna, T. gondi DNA was detected in the specimen and is hypothesised to have a role in pathogenesis
Mizuki N *et al* [66]	Case series	96 eyes	Behcet's disease	HLA-B*51 allele analysis by the PCR-sequencing based typing in Japanese population	High incidence of the HLA-B51 antigen in the Japanese BD patient group was mostly caused by the significant increase of the HLA-B*5101 allele.	B*51 allelic distribution in Japanese was different from those in Italian and Saudi Arabian populations
Murray	Case	22 eyes	Fuch's	Molecular analysis of	Predominant T-cell–	Systemic immunosuppressive

PI *et al* [67]	series		Heterochromic Iridocyclitis, Behcet's disease, Idiopathic panuveitis, Idiopathic Chronic anterior uveitis	resolving immune responses in uveitis using RT-PCR and radiometric analysis	mediated inflammatory response, involving a T_H1-like cytokine profile with expression of interleukin-2 (IL-2) and interferon alpha (IFN-α).	therapy used prior to surgery in some patients and/or the chronicity of the uveitis can suppress/switch off macrophage function, leading to resolution of T cell activity.
Quentin CD ey al [68]	Case series	52 eyes	Fuch's Heterochromic Iridocyclitis	Rubella virus antibodies and genome in aqueous humor by nested PCR in FHIC	The rubella genome was detected in 5 (18%) of 28 aqueous humor samples investigated, or in 5 (56%) of 9 patients aged <40 years.	The virus etiology is more associated in younger patients gives a rationale for omitting the ineffective corticosteroid therapy of FHC.

Conclusion

Molecular biology associated diagnostic methods have have made excellent progress in the recent past with excellent applications in medical science. The discovery of Polymerase Chain Reaction (PCR) introduced a technological advancement that is relevant for the detection of microorganisms, increasing the sensitivity, precision and accuracy of the diagnosis. Hence, has been of tremendous benefit to public health as it helps in prompt diagnosis and avoids undue delay in appropriate treatment. Although PCR is not a replacement to microscopy, culture and immunologic tests but is definitely a helpful adjunct for the diagnosis of both infectious and non-infectious uveitis.

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