Molecular Techniques in Diagnosis of Ocular Parasitic Infections

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The important ocular parasites are the protozoans Toxoplasma gondii, Acanthamoeba and Microsporidia in the order of frequency parasites causing ocular infections. The other important ocular infections caused by helminths are the larval forms of Taenia solium (Cysticercus cellulosae) and Toxocara canis. Diagnosis based on clinical appearance and serology is not always easy and is not definitive. Rapid diagnostic techniques need to be developed for direct detection of the parasitic genomes by specific molecular biological techniques especially for the helminthic infections. Molecular methods do not depend on an immune response and allow direct detection of the parasite in biological samples.

Ocular toxoplasmosis is an important disease, and progression and severity of the disease depend on the immunological status of the host, but recent studies suggest that the genetics of the parasite can also play a major role. Real-time PCR is very sensitive and is a promising technique that is capable of providing a quantitative result. Molecular methods are also used for genotypic characterization of T. gondii isolates. Analysis of polymorphic sequences determines the precise strain. However, current PCR-based assays are often labour-intensive and not readily quantifiable and have the potential for contamination due to a requirement for post-amplification sample handling. Real-time PCR can address these limitations. Multicopy sequences specific for T. gondii, e.g. the B1 gene, 529bp product, the SAG2 gene bradyzoite-specific genes (SAG-4, MAG-1) are the main target genes for development of molecular diagnostics.

Polymerase chain reaction (PCR) represents a major breakthrough for the diagnosis of infectious diseases. PCR-based methods have advantages over traditional methods for the diagnosis of toxoplasmosis, especially when serology fails and clinical symptoms are not evident or with overlapping symptoms. Rapid advances in the accuracy and rapidity of automated DNA sequencing technology, and the increasing application of automated sequencing facilities around the world, makes the use of PCR and DNA sequencing for detection and identification of microbial isolates increasingly available. The development and adaptation of new technologies for the genetic characterization and identification of parasites continue to accelerate, providing an increasing number of research and analytical tools. We review emerging technologies that have applications in this area, including real-time PCR and microarrays, and discuss the fundamental principles of some of these technologies and how they are applied to characterize parasites. We give special consideration to the application of genetic data to biological questions, where selection of the most appropriate technique depends on the biological question posed by the investigator.

Seroprevalence of toxoplasmosis based on the presence of anti-T. gondii IgG antibodies was 20.7% in 619 healthy voluntary blood donors. The seroprevalence was 78% in 50 Toxoplasma Retino choroiditis (TRC) patients (statistically significant) compared to 35.8% in 95 patients with intraocular inflammation other than toxoplasmosis. The results of local antibody production of anti-T. gondii antibodies by Witmer Desmonts’ coefficient (WDC) were positive in intraocular fluids of 47.9% of 48 clinically suspected TRC patients.1

In TRC patients, T. gondii DNA was detected by 1 or more of the 5 nested PCRs (nPCRs) employed in this study in 46% of 50 TRC patients. The clinical diagnosis of ocular toxoplasmosis was supported by laboratory tests–WDC and nPCR in 76% of 50 TRC patients including 87.5% of 16 HIV-positive TRC patients. Both tests are reliable techniques for diagnosis of ocular toxoplasmosis, but the efficacy of nPCR is superior to WDC in terms of the quantity of specimen required, rapidity, cost effectiveness and direct evidence of the presence of T. gondii in the intraocular fluid.1,2

In lens Aspirate (LA) and/or peripheral blood leucocytes (PB) of 52 congenital cataract patients, T. gondii DNA was detected in 59.6% by at least one of the 4 nPCRs—a targeting B1 gene and 2 SAG2 gene. To the best of our knowledge, this is the first report in the literature on detection of T. gondii DNA by nPCR in LA of congenital cataract patients. Genotyping of T. gondii by nPCR–RFLP and DNA sequencing analysis of B1 gene and SAG2 gene in clinical specimens of ocular toxoplasmosis patients study, revealed genotype 1 in 32 patients consisting of 11 TRC patients (including 7 HIV positive) and 21 congenital cataract patients. To the best of our knowledge, this is the first study of genotyping of T. gondii from India.3,4

Molecular biology of Acanthamoeba has advanced considerably in the last 10 years with new automated sequencing technology. This has allowed the construction of a genotype...
identification scheme with 13 different genotypes against which to compare clinical isolates for epidemiological investigations or pathogenicity markers. So far, only four genotypes have been associated with keratitis of which the majority have been T4, but T3, T6 and T11 have each caused individual cases. Each genotype is heterogeneous and can further be subdivided by comparison of sequences of diagnostic fragments of 18S rDNA, riboprinting by PCR-RFLP of 18S rDNA, or by mitochondrial DNA RFLP. The subgenus classification is based on interstrain variation in 18S rDNA sequences. The 12 different rDNA genotypes (T1–12) identified are based on sequences from 65 Acanthamoeba isolates. A 13th 18S rDNA genotype, designated ribotype T14, has recently been described.5

Ocular microsporidiosis is a group of rapidly emerging ocular protozoan infections and molecular methods for diagnosing these infections have been developed but are only at research level. However, the absence of standardized kits for commercially unattractive targets, such as most of the parasites and the fungi, has led to the development of numerous in-house PCR assays for detection of ocular infections. The performances reported both for the sensitivity and the specificity of these assays are very divergent. These shortcomings of “classical” PCR should be solved when real-time PCR assays are developed, leading to some standardization. Automated DNA extraction should also be useful to achieve this goal. Comparison between laboratories should then be possible, and regular quality controls will be necessary to ensure the reliability of real-time PCR assays.

References

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