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Use of Loop-Mediated Isothermal Amplification of DNA for the Rapid Detection of HIV/AIDS Related Opportunistic Infections (CMV &TB) in Clinical Specimens

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Abstract

This review will describe about the loop mediated isothermal amplification (LAMP) and its advantages in detection of opportunistic infections in HIV infected individuals. HIV infection is the strongest risk factor for development of tuberculosis (TB) and Cytomegalovirus (CMV) conversely both TB and CMV are the commonest HIV-associated opportunistic diseases worldwide. Loop-mediated isothermal amplification (LAMP) can amplify DNA with high specificity, efficiency and rapidity under isothermal condition. One of the great advantages of the LAMP assay is that amplification can be monitored with the naked eye using SYBR green dye. This sort of detection system is easy and helpful in discriminating infectious and non infectious samples. In view of the advantages of rapid amplification, and easy detection, LAMP has potential applications for clinical diagnosis in developing countries without requiring experienced persons and sophisticated equipment.

Keywords: Loop mediated isothermal amplification (LAMP); Human immunodeficiency virus (HIV); Opportunistic infections (OPI)

Introduction

The human immunodeficiency virus (HIV) is a retrovirus that causes AIDS (acquired immune deficiency syndrome). HIV/AIDS remains a significant problem. The concern is not only a major public health issue, but also a socio-economic and developmental crisis that affects all sectors of the population [1]. In India HIV was first identified in 1986. India is emerging globally as a country with the largest number of AIDS patients. In India, nearly 22.7 lakh people are living with HIV infection [2]. People with HIV can get many infections known as opportunistic infections or OIs. Many people do not know that they have HIV until the first time that they have an OI. Opportunistic infections are conditions that occur in individuals who have a weakened immune system. The organisms (bacteria, fungi or viruses) that cause these infections do not cause illnesses in patients who have healthy immune systems because they are able to fight off the infection. To measure the strength of the immune system of a patient with HIV is to measure the T cell count. When the T cell count is below 200 cells, the patient has developed AIDS and is at risk for opportunistic infections. The common co-infections in HIV infected individuals are tuberculosis, chronic diarrhoea, candidiasis, HSV-2, CMV, HCV and HBV. This article discusses the most common opportunistic infection TB & CMV in HIV infected individuals & loop mediated isothermal amplification (LAMP)

Mycobacterium Tuberculosis (TB)

Tuberculosis (TB) is clearly the most important severe HIV-related disease. TB infects approximately one third of the world's population and causes an estimated 2 million deaths annually [3]. TB disease in persons with HIV infection can develop immediately after exposure (i.e. primary disease) or as a result of progression after establishment of latent TB infection. An individual infected with HIV has 10 times more risk of developing TB when compared with a normal person. The risk of progression to active TB is highest in those with HIV co-infections [4]. HIV positive individual with any of the following symptoms should be suspected of having TB and investigated further: cough of more than 2 weeks duration, fever lasting more than 2–3 weeks, weight loss, fatigue, listlessness, unexplained dyspnoea and chest pain. The detection of TB among HIV individuals is very difficult.

Cytomegalovirus (CMV)

Viral infections, in particular are the main cause of HIV infected individuals and can leads to morbidity and mortality. Cytomegalovirus (CMV) disease is a frequent opportunistic infection. The virus is very common. When the immune defenses are weak, CMV can attack several parts of the body. This can be caused by various diseases including HIV. The human cytomegalovirus (CMV) is a dsDNA-virus that belongs to the β-herpesviruses (HHV-5) group [5]. Manifestation of CMV includes pneumonities, neurological diseases and retinitis. CMV retinitis is a common cause of HIV-associated blindness in this region and the second most common opportunistic infection. The most common illness caused by CMV is retinitis. It can quickly cause blindness unless treated. CMV can spread throughout the body and infect several organs at once. The risk of CMV is highest when CD4 cell counts are below 50. It is rare in people with 100 or more CD4 cells. The first signs of CMV retinitis are vision problems such as moving black spots. These are called "floaters". They may indicate an inflammation of the retina. Patients may also notice light flashes, decreased or distorted vision, or blind spots. In developing countries one third of the patients will develop CMV retinitis, in that 90% are the cases of HIV related blindness. Many doctors lack the skills for adequate management of CMV retinitis, and patients also commonly arrive to doctor when the

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disease is at late stage & finally it leads to poor outcome. Therefore there is a need for simple system for the management of CMV at the primary level.

Molecular Detection of Opportunistic Infections

Opportunistic Infections in HIV infected individuals present challenges to both early diagnosis and satisfactory management. Novel nucleic acids amplification methods are developed in order to meet these challenges. Molecular techniques such as polymerase chain reaction (PCR) can be used to solve that type of problems and increase sensitivity and specificity of pathogen detection. A PCR reaction typically utilizes two oligonucleotide primers, which are hybridized to the 5 and 3 borders of the target sequence, and a DNA polymerase, which can extend the annealed primers by adding on dNTPs to generate double-stranded products. By raising and lowering the temperature of the reaction mixture, the two strands of the DNA product are separated and can serve as templates for the next round of annealing and extension, and the process is repeated [6]. In addition to conventional PCR, recent advanced technologies, like nested PCR and real-time PCR, have been used for early and rapid detection of infections in HIV patients. Although nested PCR and real-time PCR are beneficial, they both require expensive equipment, as well as a huge amount of space in routine diagnostic laboratories, limiting their use to highly sophisticated facilities. Despite the availability of numerous diagnostic methods, there is no single rapid, sensitive inexpensive and less laborious method for field diagnosis of those diseases. Although PCR techniques have significantly increased our ability to detect infections, their requirement for a high-precision thermal cycler has prevented these powerful methods from being widely used in the field or by private clinics as a routine diagnostic tool. These methods can be technically difficult, and they require considerable expertise, which can be a major hindrance in providing correct diagnosis of the patient.

Mediated isothermal amplification (LAMP) assay

Loop Nucleic acid amplification is one of the most valuable tools in virtually all life science fields, including application –oriented fields such as clinical medicine, in which diagnosis of infectious diseases, genetic disorders and genetic traits is particularly by this new technique in addition to the widely used PCR based detection. Novel developments in molecular diagnostic tools have demonstrated the possibility of DNA amplification under isothermal conditions, i.e. without thermal cycling. A recently developed method termed loop-mediated isothermal amplification (LAMP) can amplify DNA with high specificity, efficiency and rapidity under isothermal condition [7]. Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technology that amplifies DNA under isothermal condition. The LAMP method requires a Bst DNA polymerase and set of four to six specific designed primers that recognize a total of six distinct sequences of the target DNA and with strand displacement activity.

The LAMP operation is quite simple. The target gene and the reagents are incubated at a constant temperature between 60-65°C. One of the LAMP primers can anneal to the complimentary sequence of double stranded target DNA, then initiates DNA synthesis using the DNA polymerase with strand displacement activity, displacing and releasing a single stranded DNA. This serves as a template for DNA synthesis primed by the second inner and outer primer that hybridize to the other end of the target, which produce a stem-loop DNA structure. In subsequent LAMP cycling one inner primer hybridizes to the loop on the product and initiate displacement DNA synthesis yielding the original stem-loop DNA and a new stem- loop DNA with a stem twice as long. The cycling reaction had the ability to accurately amplify a few copies of DNA to 109 in less than an hour under isothermal conditions with great accuracy. The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops. The LAMP method is also a highly efficient amplification method that allows the synthesis of large amounts of DNA in a short time. Visual detection was performed with the naked eye using SYBR green I, which turns green in the presence of amplified DNA. A sample was considered positive when the reaction mixture turned green after the addition of SYBR green I dye. The endpoint determination for a positive sample by agarose gel-based detection is done by observing a typical ladder pattern. Mori et al. [8] originally reported that the LAMP amplicons can be detected by confirming the presence of magnesium pyrophosphate, a white precipitate generated as a by-product during the reaction. Although this is a quite simple approach, detecting a small amount of the white precipitate by the naked eye is not always easy; therefore, the detection limit is apparently inferior to that of electrophoresis.

LAMP advantages

LAMP method has high specificity towards the template sequences. This is caused by recognizing the template sequences by 6 independent sequences in the initial stage, and by 4 independent sequences during later stage of LAMP reaction – partly reducing the main problem that accompanies all methods of amplification. One more advantage is the possibility of using this method for highly efficient amplification of RNA, if it is joined with reverse transcription. One of the great advantages of the LAMP assay is that amplification can be monitored with the naked eye using SYBR green dye. This sort of detection system is easy and helpful in discriminating infectious and non infectious samples, and another advantage using LAMP is based on the fact that the amplification from stem-loop structures leads to accumulation of large amounts of products of varying lengths ultimately making detection of amplified DNA much easier. Furthermore, the by-product of the reaction, magnesium pyrophosphate, is a white-colored precipitate easily seen by the naked eye.

Studies carried out on LAMP Assay in Research Group: A survey of the literature shows that the LAMP method has already been developed and applied for the detection of over 100 different pathogens [9]. Boehme et al. [10] evaluated a prototype LAMP assay with simplified manual DNA extraction for the detection of pulmonary TB in microscopy centers and demonstrated that the sensitivity of LAMP in smear- and culture positive sputum specimens was 97.7% and in smear negative, culture-positive specimens was 48.8%. The specificity in culture-negative samples was 99%. Very few studies on CMV & TB in HIV patients were reported from India. A study from Nagpur observed 88.23% sensitivity and 80% specificity compared to nested PCR which yielded 52.9% sensitivity and 90% specificity. Khushboo et al. [11] confirmed that LAMP assay is more sensitive when small mycobacterial loads are present. Other studies from Hyderabad L.V Prasad eye institute [12,13] reported 100% concordance between RT-PCR & LAMP specificity in the diagnosis of retinitis caused by CMV and HSV-1.

All the studies looked at the prevalence of opportunistic infections of HIV infected individuals and found positive for TB and CMV and most of these studies found to be detected CD4+ T-cells countless or equal to 200 cells [14,15]. Studies reported from India on viral and bacterial opportunistic infections detected in HIV infected individuals and confirmed the high association of CMV and TB with HIV infection

in small number of patients, and studies are not taken into consideration about clinical profile of the participants and other socio demographical factors associated with diseases outcome.

Conclusion

Opportunistic infections continue to be one of the most common complication of HIV infected patients and the public health. There is an urgent need to interpret the scientific findings into sustainable prevention programmes and improve public health policy. LAMP assay is an easy operation with high sensitivity and specificity, it will be simple enough to use in well-equipped laboratories with adequate bio-safety arrangement if facilities such as sample preparation, nucleic acid extraction, and cross-contamination controls are addressed. It has a great potential to improve the clinicians' ability to diagnose opportunistic infections in HIV infected individuals. More research on the effectiveness of less expensive interventions needs to be done in resource poor settings like India.

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