LOOP MEDIATED ISOTHERMAL AMPLIFICATION DETECTION OF CANDIDA ALBICANS IN BAL SAMPLES OF SUSPECTED TUBERCULOSIS PATIENTS

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(Accepted 10 October 2014)

ABSTRACT : With the increase in patients suffering from immune deficiency infections also increased pulmonary fungi and even in people in defect immune system caused fatal and lethal candidiasis. The timely diagnosis of pulmonary candidiasis is one of the problems has been detected. The purpose of this study correct and rapid diagnosis molecular Candida albicans in the samples of bronchoalveolar lavage (BAL) that can help measure suitable treatment. LAMP test Çoptimized on the basis of alfa INT1 gene and then limited of detection (LOD) and specificity evaluated. Samples were the bronchoalveolar lavage suspected of tuberculosis reviews for TB disease negative have been reported. DNA extraction carried out by standard phenol/chloroform method on samples and LAMP test was done. In the specificity test with selected primers no product was not observed with different DNA samples, that indicating the high specificity of the primers and the LOD of LAMP tests was found to be approximately 100 CFU. The review of the 50 samples that who were negative for tuberculosis, 6 cases (12%) by the LAMP test was positive for Candida albicans. LAMP test is a rapid, sensitive and specific method and also low cost for diagnosis of systemic candidiasis in samples such as BAL in laboratory centers.

Key words : LAMP, Candidiasis, Candida albicans, molecular diagnosis.

INTRODUCTION

Candida albicans is an opportunistic pathogen, which is polymorphic and is the most common cause of candidiasis (Yazdanparast, 2007; Denning et al, 1991). This fungus can be acute, subacute or chronic depending on the nature of the host, that it creates several groups infections: Superficial infections such as: oral, vaginal. candidiasis volume seen in healthy subjects (Mannarelli et al, 1998) Deep infections such as pulmonary, digestive, urinary and candidaemia occurs in patients with severe immune dysfunction (Karahan et al, 2004). These yeasts are usually opportunistic organisms (AL-Abid et al, 2004). Inhibitor of the immune system such as cancer chemotherapy, radiation therapy, the use of broad-spectrum antibiotic medication, AIDS, diabetes and causes of these fungi are aggressive invasive candidiasis is associated with a common and potentially fatal complication of cancer chemotherapy, patient’s lungs One of the most important organs involved. So that the were performed autopsies of patients candidiasis, pulmonary involvement is observed in most of them and almost half of the patients were the most commonly caught (Oh et al, 2000; Kontoyiannis, 2002) clinical signs Pulmonary candidiasis are nonspecific and mortality it was noticeable (Platenkamp et al, 1987; AL-Abid et al, 2004) one of mistake in diagnosis of pulmonary candidiasis. It is tuberculosis disease. Tuberculosis is one of chronic and contagious infectious disease that most cases is caused by Mycobacterium tuberculosis that the lung is the most common site of occurrence (Fair et al, 2007). This disease is the most common fatal disease caused by a microbe in the world. Each year, nearly 9 million people are infected with TB (Harries and Dye, 2006) and every year, nearly 2 million deaths from the disease occur. TB is a public health point of view is important in human societies. Convenience and frequency of dispersion the causative agent of tuberculosis, especially in environments that are not under the control of tuberculosis, the disease can turn into an important disease (World Health Organization, 2008). It is sufficient to describe the 20 million people with TB in the world, and Each year, 5 million people will be added to this cultivar. The most disadvantaged areas of the world are victims.
of tuberculosis. The majority of pulmonary candidiasis, candida infections caused by Candida albicans. While the use of new antifungal drugs, go to work repairing the control of fungal infections has been a major problem in the early diagnosis of infection remains. So start early treatment is in reducing the mortality rate of patients with immune suppression, an important criterion. Final diagnosis obtained by showing invasion in tissue sections. But lung biopsy it is not possible in many cases (Sarosi and Davies, 2000). The old instruments identification of candidate included: morphological, absorption test, identification of isolates in culture. Several days are time required and when using automatic biochemical systems, clinical yeast isolates are sometimes misidentified. molecular methods detection is based on the isolation and amplification of nucleic acids that Including is polymerase chain reaction methods and isothermal amplification. That even in the early stages hybridization Contaminations are to detect impurities accurate (Vernet, 2004; Shahhosseiny, 2005). Each amplification methods Despite the strengths with its own problems, due to the use of advanced equipment such as thermocycler Can not be used publicly in all diagnostic centers (Nagamine, 2002; Zhang et al, 2001) loop-mediated isothermal amplification (LAMP) Techniques is another method of isothermal amplification In which amplification Be done manner isothermal. And therefore do not require a thermocycler. In addition, is the accuracy and sensitivity (Anaissie et al, 2003, Sarosi and Davies, 2000) This reaction is done without template DNA denaturation and whit using the polymerase with the ability to substitution in the chain. Also is used of 6 specific primers that known as internal FIP(F1,C,F2) andBIP,(B1,C,B2)and external(F3,B3) (Special Loop LFL,B) primers that they have very high specificity (Vernet, 2004; Shahhosseiny, 2005). FIP primer on the template DNA strand binds to the region complementary(F2C) the template strand and Complementary strand begins synthesis. The substitution of external primers F3, B 3 and using the Bst polymerase is a long DNA string Which leads to the Is produced dumbbell DNA Structure that quickly with DNA synthesis is converted into DNA structure stems - loop. The structure of the DNA stems - loop acts as a starter LAMP cycle (Fair et al, 2007, Harries and Dye, 2006) With special probe design of these structures, they can be used in hybridization (Anaissie, 2003; Fair et al, 2007) In this case there is no need to use heat denaturation after amplification This means that all stages of amplification to detect is performed at a temperature. Also when the reaction was accompanied with reverse transcription of RNA sequences can also be reproduced (Zhang et al, 2001) So developed molecular methods provide for rapid detection feels more accurate than The traditional and old phenotypic methods and also for epidemiological studies and molecular analysis of an infectious agent is important for infection control. The purpose of this research was to rapid detect molecular by LAMP method To determine the presence of Candida albicans in samples suspected to tuberculosis disease. Which ultimately leads to an accurate and rapid diagnosis at the same time, however, can do all diagnostic centers for early detection of Candida albicans. Which ultimately is resulted in an accurate and rapid diagnostic method however, can be done in all of the diagnostic centers for early detection with low cost of Candida albicans.

**MATERIALS AND METHODS**

Within a few months in one study of patients referred to a specialized TB laboratory in Tehran that showed specific symptoms of TB patients, 50 people who were randomly selected TB patients were negative Sampling was done by a pulmonary specialist with Bronchoalveolar lavage (BAL) sample were prepared using standard. Samples were collected in sterile tubes and were transported to the laboratory as soon as possible.

**Preparation strains of Candida albicans and culturing methods:** First, lyophilized and standard strains of Candida albicans belonging to Iranian Industrial Bacteria and Fungi Collection (Persian Type Culture Collection, PTCC) were cultured at GYEP liquid medium.

After the organism growth, 500 µl of the liquid medium was removed and centrifuged at 12000 rpm for 5 min. The supernatant was discarded and resulting precipitation was deionized in 100 µl of sterile double-distilled water and suspended; then, its DNA was extracted using phenol-chloroform methods (Sambrook and Russell, 2006).

**DNA extraction**

**Extracting DNA from standard strain:** Phenol-chloroform method was used for DNA extraction (Sambrook and Russell, 2006).

100 µl of the cultured strain in the liquid medium was removed; then, at the first stage, 500 µl of the lysis solution or buffer lysis (Proteinase K= 250 µg/ml, Tris-Hcl=50 Mm, SDS = 10%) was added.

At the second stage, 10 µl protease was added and shaken for 10 sec.

At the third stage, it was put in a 65° heater block for 20 min. After removal of the solution inside the tube, phenol-chloroform solution was added and, after 10 times of inversion, it was centrifuged for 5 min. Afterward, the tube was placed on ice for 30 min. After removing from
Isothermal amplification detection of *Candida albicans* in BAL

Figure 1: LAMP Optimized Test
Tube 1: gene-specific amplification of *Candida albicans*
Tube 2: negative control

Figure 2: Results of LOD test.
Tube 1: Positive control
Tube 2: Dilution of 10^-1 equivalent to 1,000,000 copies of DNA, *Candida albicans*
Tube 3: Dilution of 10^-2 equivalent to 100,000 copies of DNA, *Candida albicans*
Tube 4: Dilution of 10^-3 equivalent to 10,000 copies of DNA, *Candida albicans*
Tube 5: Dilution of 10^-4 equivalent to 1000 copies of DNA, *Candida albicans*
Tube 6: Dilution of 10^-5 equivalent to 100 copies of DNA, *Candida albicans*
Tube 7: Dilution of 10^-6 equivalent to 10 copies of DNA, *Candida albicans*
Tube 8: Dilution of 10^-7 equivalent to 1 copy of DNA *Candida albicans*
Tube 9: negative control

Figure 3: Specificity test.
Tube 1: DNA of *Candida albicans*, Tube 2 to 7: DNA *Cryptococcus neoformans*, *Fusarium spp*, *Fusarium solani*, *Aspergillus parasiticus*, *Escherichia Coli (E. coli)*, *Hepatitis B virus (HBV)*, Tube 8: negative control

ice, the supernatant was transferred to a new tube and some isopropanol with the same volume of the solution was added to the tube. After 10 times of inversion, it was put in a -20°C freezer for 10 min. Once taken out of the freezer, it was centrifuged for 10 min at 12000 rpm and the supernatant was discarded; because isopropanol always causes DNA precipitation, the supernatant lacks any DNA. Afterwards, 1000 µl of alcohol 70% was poured on it and, after 10 times of inversion, centrifuged for 10 min at 12000 rpm (alcohol separates isopropanol from DNA). Finally, the supernatant was decanted (discarded), the test tube was placed in the 65°C heater block, and 100 µl distilled water was added.

**Primer design**: LAMP primers using Primer explorer V4 software was designed for gene alph-INT1. Specific primers of *Candida albicans* are as shown in...
The LAMP reaction was made in 25 µl by mixing 0.2 µM F3/B3, 1.6 µM FIP/BIP, 20 Mm Tris-Hcl, 10 Mm Kcl, 10 mM (NH₄)₂SO₄, 9 mM MgSO₄, 1.4 mM dNTP, 0.8 M Betain (Sigma- Aldrich), 8 u Bsm DNA polymerase (New England Biolabs). Themixture was incubated at 61 for 1 h.

Determine the limit of detection LOD and specificity of the LAMP test: In order to determine the LOD of these techniques in this research, the standard strain of Candida albicans was applied in preparing a series of dilutions from 10 million copies to five copies of DNA to LAMP reactions.

Test specificity

DNA from different microorganisms such as Cryptococcus neoformance, Fusarium spp, Fusarium solani, Aspergillus parasiticus, Escherichia coli (E. coli), Hepatitis bivrus (HBV), human DNA with the DNA of the fungus Candida albicans and specific primers for fungus placed in tube different and was performed LAMP test.

LAMP test results

On 50 BAL samples from suspected tuberculosis patients tested, 6 were positive for Candida albicans That 12 percent of those who had been admitted with symptoms of tuberculosis were diagnosed with pulmonary candidiasis (Figure 3).

DISCUSSION

Opportunistic infections caused by yeasts in recent decades has been of great importance (Pfaller, 1995) This is due to the increasing incidence and prevalence of these infections in the community and nosocomial infections. Debilitating diseases such as AIDS, diabetes and cancer, and There is the use of catheters, organ transplants, cancer drugs, broad-spectrum antibiotics and Corticosteroids treatment of yeast infections is the subject of resolutions predisposing (Perlroth et al, 2007). Invasive candidiasis is a common and potentially fatal side effects caused by treatment, especially chemotherapy, cancer patients Candida species are the most common cause of fungal infections in humans (Anaissie et al, 2003; AL-Abeid, 2004) , so that candidiasis fungal is included 66-80% of fungal infections . The incidence of disseminated candidiasis in 1990 was approximately 80% of all cases of candidaemia due to Candida albicans. Recent reports show from other countries, a change in the epidemiology of candidiasis (Perlroth et al, 2007, Fanello et al, 2006).

This study primer the desired is designed from sequences encoding gene alph-INT1 Candida albicans. This gene is similar to vertebrates leukocytes eynstren (Marcilla et al, 1999) this primer in terms of specificity with Candida spp, Cryptococcus neoformance, Fusarium spp, Fusarium solani, Aspergillus parasiticus E. coli, Escherichia coli, Hepatitis bivrus (HBV) were tested, and just alph-INT1 attached and doing Proliferation. The
study and other studies. Pulmonary candidiasis in cancer patients is a usual infection, Candida species colonize is not uncommon more than one event at a specific place. As in the present study, 12% colonization rate in patients who were examined for tuberculosis were reported and has increased other species such as C. glabrata and C. tropicalis, C. dubliniensis, C. parapsilosis, C. krusei and C. lvsytanya (Akpan A. and Morgan R., 2002). Increased pulmonary candidiasis was associated with widespread use of therapy azole, prophylaxis and fluconazole (Pfaller et al, 1999) is necessary accurate and rapid identification of Candida to antifungal therapy effective, it can detect the possible in a few hours because no need to cultivate in molecular methods. Another advantage is sensitive of this method that a few yeast cells can be proceed to detect (Kochl et al, 2005). LAMP method is very simple of gene amplification method that given temperatureof beginning to the end. LAMP method is used for detection, identification and isolation candidiasis of other fungi. This method is simple and also has a very high sensitivity and accuracy. And also is performed at low cost because it does not need to device and hardware like thermocycler (Notomi et al, 2000). The LAMP method for rapid detection of Candida albicans, is designed two pairs of primers on the basis of alph-INT1 gene that is able to detect Candida albicans and diagnosis is done very rapid and accurate (Maeda et al, 2005). This method has many advantages and The method is amplification DNA at a certain temperature and in a short time And can be started diagnosis with a few DNA Reason High Sensitivity in this method is highly specific Amplification of target genes because is able to with 4 primer at the beginning of the reaction have been identified 6 region sequences of the target gene and during the reaction 4 region. This technique does not require advanced facilities and the reaction is done by using a simple Dry Plate Reaction verification was with the addition of cyber-lamp and observed under UV light easily without having to electrophoresis in a very short time. Molecular methods have helped to rapid and accurate detect of Candida albicans Rapid and available detection Candida albicans and timely treatment of patients be prevented from rising costs patients the results of this study are help to remove disease and avoid increasing costs to patients for early diagnosis. Given that the departments of molecular diagnostic have higher speed and accuracy toward tests based on phenotype diagnostic tests based on phenotype. Other researchers have shown priority this method over the frequently asked methods and also in clinical laboratories Replace methods are frequently asked methods and used routinely. So it can be stated with the help of this techniques may be provided molecular diagnostic without for advanced equipment with a minimum price and with high accuracy and sensitivity.

**CONCLUSION**

LAMP test is a rapid, sensitive and specific method and also low cost for diagnosis of systemic candidiasis in samples such as BAL in laboratory centers and also contamination with these fungi was high in treated TB or TB-suspicious patients, physicians should pay particular attention to this issue and avoid unnecessary treatments without definitive diagnosis.

**REFERENCES**


