Comparative Analysis of Quantitative Real-time PCR and Agglutination Detection in Candida Species Isolated from Clinical Urine Specimens of Old Patients

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Abstract

Objective: The study aimed to aim to detect the type of Candida species isolated from the urine samples of older patients.

Methods: We collected the urine samples of 102 older patients. We divided the patients into 2 groups (the control group and the experimental group) randomly. The control group used the agglutination detection. The experiment group used the Quantitative Real-time PCR detection. We detected and analysis the data in the groups.

Results: The positive ratio of the experimental group was 80.39 % (41/51), on the other side, the positive ratio of the Experiment group was 68.62%(35/51). The positive ratio of the experimental group was higher than the control group (P<0.05). The detect ratio of *Candida albicans* of the experimental group and control group was 53.5% and 51.4%.

Conclusion: Our finding demonstrated that the Quantitative Real-time PCR detection method was more effective than the agglutination detection in the urine sample of older patients.

1. Introduction

The Candida species opportunistically induced the disease in the people whose defense mechanisms were damaged. The pathogenic Candida species might induce the diseases ranging from superficial mycoses to fatal effect. The incidence of Candida-related infections has increased steadily over the past three decades [1].The infection ratio of Candida species has raised in the past 30 years, which might be due to medical therapy and the increasing immunocompromised patients with antibiotic intervention, AIDS and cancer [2-3] .Furthermore, the Candida species infection increased because the application of medical device such as heart valves, pacemakers, catheters and joint replacements. These implants became colonized by microorganisms, which produced biological membrane and induced invasive disease [4].Previous reports identified the pathogenic factors of Candida species infection to ease the diagnosis and cure of candidacies [5-8]. The pathogenic factors of Candida species infection were including the phenotypic variability, adherences to host tissue, toxins and enzymes. In the present study, we want to assess pathogenic Candida species isolated from various clinical specimens of old patients, therefore, we could provide useful information for clinical therapy.

2. Methods

2.1 Samples

We collected the urine samples of 102 older patients in The First Affiliated Hospital of Chongqing Medical University from March 2017 to September 2017.We divided the patients into 2 groups (the control group and the experimental group) randomly. There were 27 females and the 27 males in the control group (the average age was 71.8±6.5). Otherwise, there were 27 females and the 27 males in the experimental group (the average age was 72.4±6.9).

2.2 Detection method

The control group used the agglutination detection. We performed the experiment according to the standard protocol. If the purple particle occurred, the result showed positive, which demonstrated the Candida species existed in the urine sample. If the purple particle did not occur, the result showed negative, which demonstrated the Candida species did not exist in the urine sample. The experiment group used the Quantitative Real-time PCR detection. The prime is 5-TTCACATATGGAAGCCAGAGGG-3;5-CAATGGCACAAAGGAATCCAGC-3. The PCR On the basis of the manufacturer's instructions (Roche ,Swiss). Quantitative Real-time PCR was performed in a 20μl reaction mixture, 10μl PCR premixed solution, 0.8μl thermus aquaticus (Taq) and 5μl primer mixture. Amplification was carried out by initial denaturation at 95°C for 5 min followed by 5 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30s and elongation at 72°C for 30s. In total, 40 cycles were conducted.

2.3 Statistical analysis

All analyses were carried out using the SPSS 17.0 statistical software. Pearson's $\chi2$ test was performed to evaluate the significance of differences between designated groups. All analyses were two-sided and interpreted as being significant at $P<0.05$.

3. Results

In the Table 1, there were 28 female and 23 male in the Experiment group, on the other way, there were 26 female and 25 male in the control group. There were no difference between the groups (P>0.05).There were 15 patients with cardiovascular and cerebrovascular diseases, 13 patients with diabetes mellitus, 9 patients with tuberculosis, 4 patients with liver cirrhosis, 8 patients with cancer in the experiment group , which did not have no difference with the control group (Table 2). The Table 3 demonstrated that the positive ratio of the experimental group was 80.39% (41/51), on the other side, the positive ratio of the experiment group was 68.62% (35/51).The positive ratio of the experimental group was higher than the control group (P<0.05). The Table 4 demonstrated that the detect ratio of *Candida albicans, C. glabrata, C.tropical, C. krusei* was 53.5%, 21.9%, 12,1%,4.8%,7.3% in the Experiment group ,respectively. The detect ratio of *Candida albicans, C. glabrata, C.tropical, C. krusei* was 51.4%, 22.8%, 11.4%, 8.5% in the control group, respectively.

Group	Female	Male
Experiment group	28	23
Control group	26	25

Table 1: The gender distribution in the Experiment groups and Control group

Group	Cardiovascular and cerebrovascular diseases	Diabetes mellitus	Tuberculosis	Liver cirrhosis	Cancer	Other disease
Experiment group	15	13	9	4	8	2
Control group	16	14	7	3	7	4

Table 2: The disease distribution in the Experiment groups and Control group

Group	Positive number	Positive ratio(%)
Experiment group	41	80.39(41/51)
Control group	35	68.62(32/51)

Table 3: The Positive samples in the Experiment groups and Control group

Group	n	*Candida albicans*	*C. glabrata*	*C.tropical*	*C. krusei*	Other Candida
Experiment group	41	22(53.5%)	9(21.9)	5(12.1)	2(4.8)	3(7.3)
Control group	35	18(51.4)	8(22.8)	4(11.4)	3(8.5)	2(5.7)

Table 4: The Candida type distribution in the Experiment groups and Control group

4. Discussion

Candida species have existed in the world widely, but in the human body, the highest content of Candida species existed in the digestive tract, then the vagina. In the normal condition, the multiple floras were in the balance, which did not induce disease. But when the immunity of the human body was decreasing, or the pH value of some organs changed, the balance of flora was interrupted, therefore, the Candida species invaded in the human body and induced the disease.

When the organ function and body immunity were decreasing, older patients were more likely to be invaded by the Candida species. Moreover, the widely application of antibiotics and hormone drug induced the occurrence of the multiple drug-resistant bacteria, which interrupted the balance between the bacteria group and fungus group, enhanced the infection ratio of Candida species.

We should detect Candida species using accurate method and then use the precise drug targeted different type of Candida species. In our study, the positive ratio of Quantitative Real-time PCR detection was higher than the agglutination detection ($P<0.05$). Moreover, the Quantitative Real-time PCR detection method was more effective and time-saving method. Many other factors like surface recognition molecules, hyphal switching, extracellular hydrolytic enzymes and phenotypic switching have been involved in pathogenicity of Candida species [9]. Mattei et al. demonstrated phospholipase activity was detected for 78% of *C. albicans* isolates, and proteinase activity was detected for 97% of isolates from bloodstream samples [10].

Our finding demonstrated that the Quantitative Real-time PCR detection method was more effective than the agglutination detection. The present study aimed to detect the type of Candida species isolated from the urine samples of older patients. But the type detection ratio between the two methods have no difference. A wide range of clinical samples should be studied in the future. The pathogenicity of Candida species were still needed to be validated.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Madhavan P, Jamal F, Chong PP. Laboratory isolation and identification of Candida species. J Appl Sci 11 (2011): 2870–2877.

2. Karkowska-Kuleta J, Rapala-Kozik M, Kozik A. Fungi pathogenic to humans: molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus. Acta Biochim Pol 56 (2009): 211–214.

3. Jahanshiri Z, Manifar S, Moosa H, Asghari-Paskiabi F, Mahmoodzadeh H, Shams-Ghahfarokhi M, et al. Oropharyngeal candidiasis in head and neck cancer patients in Iran: species identification, antifungal susceptibility and pathogenic characterization. J Mycol Med 2018.

4. Hawser SP, Douglas LJ. Biofilm formation by Candida species on the surface of catheter materials in vitro. Infect Immun 62 (1994): 915–921.

5. Yang Y. Virulence factors of Candida species. J Microbial Immunol Infect 36 (2003): 223–228.

6. Razzaghi-Abyaneh M, Sadeghi G, Zeinali E, Alirezaee M, Shams-Ghahfarokhi M, Amani A, et al. Species distribution and antifungal susceptibility of Candida spp. isolated from superficial candidiasis in outpatients in Iran. J Mycol Med 24 (2014): 43–50.

7. Sadeghi G, Ebrahimi-Rad M, Mousavi SF, Shams-Ghahfarokhi M, Razzaghi- Abyaneh M. Emergence of non-Candida albicans species: epidemiology phy-logeny and fluconazole susceptibility profile. J Mycol Med 28 (2018): 51–58.

8. Amanloo S, Shams-Ghahfarokhi M, Ghahri M, Razzaghi-Abyaneh M. Genotyp-ing of clinical isolates of Candida glabrata from Iran by multilocus sequence typing and determination of population structure and drug resistance profile. Med Mycol 56 (2018): 207–215.

9. Mohan das V, Ballal M. Proteinase and phospholipase activity as virulence factors in Candida species isolated from blood. Rev Iberoam Micol 25 (2008): 208–210.

10. Mattei AS, Alves SH, Severo CB, Guazzelli Lda S, Oliveira Fde M, Severo LC. Determination of germ tube, phospholipase, and proteinase production by bloodstream isolates of Candida albicans. Rev Soc Bras Med Trop 46 (2013): 340–342.

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