Int J Pharm Bio Sci 2014 April ; 5 (2) : (B) 36 - 40

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPARATIVE ANALYSIS OF REAL TIME PCR AND ELISA FOR THE DETECTION OF *PSEUDOMONAS AERUGINOSA* IN NOT CHRONICALLY INFECTED CYSTIC FIBROSIS PATIENTS

MAHSHID KHODABAKHSHI, SELVAM ARJUNAN, KHALID IMRAN AND RADHAKRISHNAN SENTHILKUMAR*

Indian Academy Degree College, Hennur cross, Bangalore 560043, India

ABSTRACT

Detection of *Pseudomonas aeruginosa* using the serologic test have been found useful for differentiating colonization from infection, particularly in chronic disease. A quantitative polymerase chain reaction (qPCR) was compared with the Enzyme linked immunosorbent assay (ELISA) used routinely. The qPCR detected the targeted gene of *P. aeruginosa* outer membrane proteins, whereas the ELISA reacted with IgGs against soluble *P. aeruginosa* antigens. Among the 37 sera from the patients with not chronic lung disease studied, all those with ELISA reactivity were positive by qPCR. Thus, qPCR is more sensitive and specific than the ELISA, suggesting that the former technique may be useful for the early diagnosis of infection.

KEYWORDS: ***Pseudomonas aeruginosa*, ELISA, Real Time PCR, Cystic Fibrosis**

RADHAKRISHNAN SENTHILKUMAR
Indian Academy Degree College, Hennur cross, Bangalore 560043, India

INTRODUCTION

Pseudomonas aeruginosa is one of the major respiratory pathogen causing severe lung infections among cystic fibrosis (CF). CF is one of the most common genetic disorders, caused by mutations in the CFTR gene, coding for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein[1]. It is associated with increased morbidity and mortality[2]. It is well established that early antibiotic treatment is able to postpone the transition to chronic lung infection. As a result, it is crucial to detect new infection with *P. aeruginosa* at the earliest in an attempt to eradicate the bacterial colonization as early as possible[3]. At present, however, routine detection and identification of *P. aeruginosa* in respiratory samples is done by conventional methods such as culture and biochemical characteristics, diagnostic mistake can occur due to the variable phenotypic characteristics of this species. Besides, the sensitivity of culture might be restricted, in particular, as compared to DNA amplification based techniques[4, 5]. Thus, the serologic tests for *P. aeruginosa* have been found useful for differentiating colonization from infection, especially in chronic disease. In the present study, in order to optimize the early detection, we compared the sensitivity of the quantitative PCR (qPCR) with the ELISA used routinely for the detection of *P. aeruginosa* in respiratory samples of not chronically infected CF patients.

MATERIALS AND METHODS

MATERIALS

Pseudomonas-CF-IgG ELISA Kit were purchased from Statens Serum Institute Denmark, Maxisorp ELISA plate (NUNC®) from eBioscience, Inc. lyophilized Pseudomonas *aeruginosa* antigen were purchased from Statens Serum Institute Denmark. Human standard antiserum and Rabbit-Anti-Human IgG HRP from MP Biomedicals (India) Pvt Ltd. Fluorescent dyes and Fluorescent probes were purchased from Life Technologies.

METHODS

Sample Collection

Patients samples were collected from Clumax clinic and plasma was stored stored at -70°C until use.

ELISA

ELISA using a kit was done on blood (serum) obtained from all patients by Erba Lisa assay method. The test was performed to detect antibodies against *Pseudomonas aeruginosa* antigen using commercially available kits according to manufacturer's instructions. Serum dilution of 1:100 was used in the assay. Positive and negative control sera were also included along with test sera for the analysis. In order to determine the antibodies in units, the curve was created by plotting the OD values of different reference sera. Thereby, the concentration of antibodies in test serum sample was studied by extrapolating the OD value of serum.

DNA-Extraction and Real Time PCR

Pseudomonas *aeruginosa* DNA is extracted from plasma using the phenol, chloroform, and isoamyl alcohol (25:24:1) extraction method as described by the manufacture instruction guide (Life technologies). For the quantitative detection of Pseudomonas *aeruginosa* from patient's samples, Real-TM Quant kit was used. Pseudomonas *aeruginosa* DNA is extracted from plasma amplified using real time amplification using primers PAO1 A gene (5′ CAGGTCGGAGCTGTCGTACTC 3′), PAO1 S gene (5′ ACCCGAACGCAGGCTATG 3′) and opr L TM gene (5′ FAM-AGAAGGTGGTGATCGCACGCAGA- BBQ 3′) was performed based on the manufactured guidance. Both *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* IC detected using fluorescent reporter dye probes. Internal Control (IC) serves as an extraction and an amplification control for each individually processed specimen and to identify possible inhibition. IC is detected in a channel other than the *Pseudomonas aeruginosa* DNA. Monitoring the fluorescence

intensities during Real Time allows the detection and quantification of the accumulating product without having to re-open the reaction tube after the real time amplification.

Statistical Analysis

All statistical analyses were performed using the student's paired t test or fisher exact probability test. Descriptive statistics including the frequency, mean, median, and standard deviation (SD) were determined.

RESULTS AND DISCUSSION

We made a comparative study of among the 37 sera from the patient samples were studied the sensitivity of the quantitative PCR (qPCR) with the ELISA used routinely for the detection of *P. aeruginosa* in respiratory samples of not chronically infected CF patients. Various age group of people were analyzed the sensitivity. Interestingly, as showed in Fig.1, all those with ELISA reactivity were positive by qPCR. Thus, qPCR is more sensitive and specific than the ELISA.

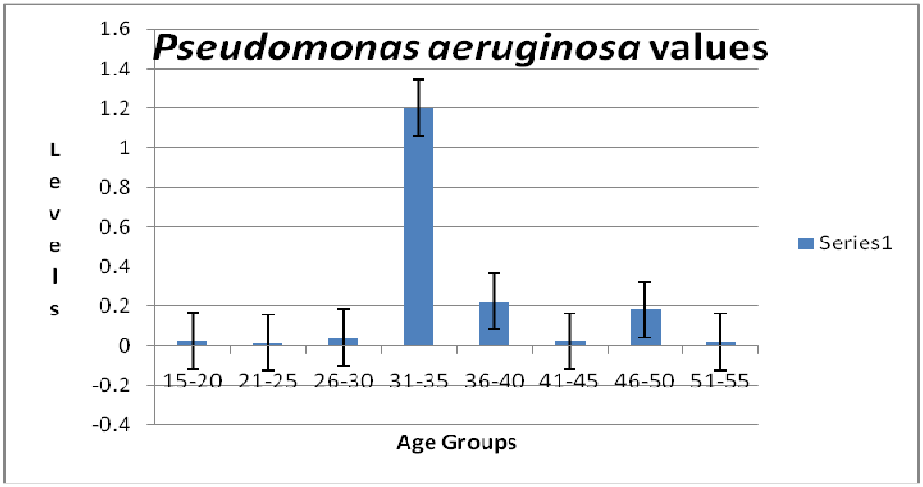

Figure 1

Graph showing the Pseudomonas aeruginosa values of different age groups based on ELISA readings (Levels - U/L). Age group of 31-35 is found to be significant. All the values are means of the triplicates.

There was amplification at 28th cycle with the gene copies reaching up to approximately 112868. The *Pseudomonas aeruginosa* gene also showed amplification significantly in the age groups 21-25, 36-40 and 46-50. The ct value confirms the gene amplification however its shows the significant amplification in at the age group of 31-35 (Fig.1).

Age group	Pseudomonas aeruginosa value	Ct value	Copies
15-20	0.024	Nil	
21-25	0.015	24	868
26-30	0.011	Nil	
31-35	1.2	38	112868
36-40	0.224	30	4638
41-45	0.022	Nil	
46-50	0.182	26	7535
51-55	0.018	Nil	

Table 1

Comparative values of the ELISA readings and Real time PCR Ct values of the Pseudomonas aeruginosa levels.

The ELISA reading shows that the significant values in the age group of 31-35. However these results further conforming that the Real time PCR values of the ELISA. Similarly, the Ct value of the gene amplified showed significant values (p> 20) in the age group 31-35, which confirms the readings of the ELISA and Real time PCR values (Table. 1). Early detection of *P. aeruginosa* has become paramount, consequently, now it is possible to postpone chronic infection with the use of early aggressive antibiotic treatment. In most routine microbiology laboratories, microbiological culture is still the mainstay for detection of *P. aeruginosa*[6]. However, other detection methods that might be more sensitive than the culture still need evaluation and validation. Nowadays, in order to detect the new infection episodes as soon as possible, serological testing for *P. aeruginosa* antibodies has been proposed as an alternative to culture. Several groups reported that anti-*P. aeruginosa* antibodies can be detected prior to *P. aeruginosa* detection by culture and prior to the onset of chronic infection7. However, in a cross-sectional study by da Silva Filho et al., found that microbiological culture or PCR is more sensitive than the serology[8]. In the current study, we evaluated whether qPCR can improve early detection of *P. aeruginosa* in respiratory samples from patients, not yet chronically infected with this organism. In the past decade, several PCR formats and other molecular methods for the detection of P. aeruginosa have been developed. Some studies found a higher sensitivity of PCR in comparison with culture and/or biochemical tests for the detection of *P. aeruginosa* from respiratory samples of CF patients[5], while others found no difference[9] or a lower sensitivity for PCR[10]. In this study, we targeted the oprL gene, previously shown to be a more sensitive gene locus than the exotoxin A locus, when applied to CF patient airway samples. In a previous study by Deschaght P et al, five DNA-extent study traction methods, six qPCR formats and three culture techniques to optimize were compared and the detection of *P. aeruginosa* in sputum from CF patients was validated[11]. Using a dilution series of *P. aeruginosa* in sputum, the three culture methods were equally sensitive to each other but also to the combination of the most sensitive DNA extraction method and the most sensitive amplification assay, i.e. probe based qPCR.

A study by Xu J et al, in which *P. aeruginosa* detection by culture and by qPCR is compared in a long term study[5]. It was concluded that PCR detected *P. aeruginosa* on average 4.5 months prior to culture. This result should be interpreted with caution, because also in the same study only 5 of the 10 culture negative, PCR positive patients became *P. aeruginosa* culture positive during the follow-up period. It can also be argued whether the cultured strain was identical as the one causing PCR positivity 4-17 months prior to culture positivity, given the long follow-up period and the fact that the average conversion rate to culture positivity among CF patients can be considered as relatively high. At last, the study also found 5 cultures positive, PCR negative samples, for which PCR might have become positive later on, however no follow-up data were reported. However, our study, based on the qPCR for *P. aeruginosa* than ELISA, may have a predictive value for imminent *P. aeruginosa* infection in only a limited number of cases.

CONCLUSION

The current study shows that the DNA amplification technique is more sensitive and specific than ELISA for detection of *P. aeruginosa* in respiratory samples of patients with chronic lung infection. However, these techniques have been performed in a small number of samples for the early detection of *P. aeruginosa*, further studies involving large number of samples are necessary to confirm the sensitivity of qPCR i.e. probe based qPCR when compared to ELISA.

REFERENCES

1. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N: Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989, 245:1059-1065.
2. Kerem E, Corey M, Gold R, Levison H: Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonization with Pseudomonas aeruginosa. J Pediatr 1990, 116:714-719.
3. Frederiksen B, Koch C, Høiby N: Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. Ped Pulmon 1997, 23:330-335.
4. Taylor RFH, Hodson ME, Pitt TL: Adult cystic fibrosis: association of acute pulmonary exacerbations and increasing severity of lung disease with auxotrophic mutants of Pseudomonas aeruginosa. Thorax 1993, 48:1002-1005.
5. Xu J, Moore J, Murphy PG, Millar BC, Elborn JS: Early detection of Pseudomonas aeruginosa - comparison of conventional versus molecular (PCR) detection directly from adult patients with cystic fibrosis (CF). Annals Clin Microbiol Antimicrob 2004, 3:21-26.
6. Vasquez C, Municio M, Corera M, Gaztelurrutia L, Sojo A, Vitoria JC: Early treatment of Pseudomonas aeruginosa colonisation in cystic fibrosis. Acta Paediatr Scand 1993, 82:308-309.
7. West SEH, Zeng L, Lee BL, Kosorok M, Laxova A, Rock MJ, Splaingard MJ, Farrell PM: Respiratory infection with Pseudomonas aeruginosa in children with cystic fibrosis: early detection by serology and assessment of risk factors. J Am Med Assoc 2000, 287:2958-2967.
8. da Silva Filho LVF, Tateno AF, Martins KM, Chernishev ACA, De Oliveira Garcia D, Haug M, Meisner C, Rodrigues JC, Döring G: The combination of PCR and serology increases the diagnosis of *Pseudomonas* aeruginosa colonization/infection in cystic fibrosis. Ped Pulmon 2007, 42:938-944.
9. Van Belkum A, Renders NHM, Smith S, Overbeek SE, Verbrugh HA: Comparison of conventional and molecular methods for the detection of bacterial pathogens in sputum samples from cystic fibrosis. FEMS Immunol Med Microbiol 2000, 27:51-57.
10. Qin X, Emerson J, Stapp J, Stapp L, Abe P, Burns JL: Use of real-time PCR with multiple targets to identify Pseudomonas aeruginosa and other non fermenting gram-negative bacilli from patients with cystic fibrosis. J Clin Microbiol 2003, 41:4312-4317.
11. Deschaght P, De Baere T, Van Simaey L, Van daele S, De Baets F, De Vos D, Pirnay JP, Vaneechoutte M: Comparison of the sensitivity of culture, PCR and quantitative real-time PCR for the detection of Pseudomonas aeruginosa in sputum of cystic fibrosis patients. BMC Microbiol 2009.