Efficacy of rapid multiplex polymerase chain reaction for early diagnosis and treatment of pertussis

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Research article

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Abstract

Background Pertussis, a highly infectious respiratory disease caused by Bordetella pertussis, causes airway inflammation and severe, persistent (lasting 2 weeks or more) characteristic whooping cough. In severe cases, complications such as atelectasis and bronchopneumonia may occur. Recently, the prevalence of pertussis has increased in South Korea due to reduced effect of the DTaP vaccination in infants as their age increased. Although culture is the gold standard test for diagnosis, polymerase chain reaction (PCR) method is most commonly used for diagnosis of pertussis due to the low sensitivity and long turnaround time of the culture method. Recently, a rapid multiplex PCR test has been introduced for comprehensive detection of respiratory pathogens (17 viruses and 3 bacteria), including Bordetella pertussis, with a turnaround time of 1 hour. This study aimed to investigate the efficacy of multiplex PCR for early diagnosis and treatment of pertussis.

Methods We performed a retrospective study on patients with pertussis diagnosed from May 2017 to June 2019 at Yeungnam University Hospital. Nasopharyngeal swab specimens were tested using multiplex PCR. Medical records collected included data on age, sex, symptoms at the time of diagnosis, admission, hospitalization, isolation, vaccination history, past medical history, and accompanying diseases.

Results A total of 27 patients were diagnosed with pertussis, nine (33.3%) of whom were men, with a median age of 48.9 years (3.3–82.2). Eleven (40.7%) had fever, 12 (44.4%) had dyspnea, 3 (11.1%) had paroxysmal cough, and 9 (33.3%) had inspiratory whooping. Seventeen (62.9%) and 24 (88.8%) patients had coughing for <8 days and ≤14 days, respectively. Median time from first symptom to diagnosis was 9.0 (1–31) days. Twenty-four patients (81.5%) were diagnosed within 2 weeks. All but one patient was prescribed macrolide antibiotics; all patients were isolated, with 22 (81.5%) requiring hospitalization. Three patients (11.1%) received ICU care for ventilation. All patients survived.

Conclusion A rapid multiplex PCR test can ensure early diagnosis, isolation, and treatment of pertussis. Testing of patients with respiratory symptom with multiplex PCR can led to early diagnosis of pertussis, proper treatment, and may help in outbreak control.

Background

Pertussis is a highly contagious disease that causes a wide range of clinical symptoms ranging from trivial to serious illnesses, resulting in death in young infants [1]. Pertussis spreads through respiratory droplets and can be transmitted by coughing, sneezing, or sharing breathing space for extended periods of time [2]. The most common side effect of pertussis is apnea followed by pneumonia, seizures, and encephalopathy. In more severe cases, pertussis can be life-threatening with refractory pulmonary hypertension and pneumothorax [1].

In South Korea, the government introduced the DTaP (diphtheria, tetanus, and acellular pertussis) vaccine in the National Immunization Program (NIP) in 1989. During 1955–2011, the secular trend in pertussis

incidence was characterized by a gradual decrease in the reported number of cases from 1955 to the late 1990s, followed by a recent increase from the early 2000s [3]. The pertussis vaccination is administered as three doses of basic vaccination with DTaP administered at 2, 4 and 6 months. The pertussis booster vaccination is administered at 15–18 months, at 4–6 years with DTaP, and at 11–12 years with Tdap. The Korean DTaP vaccination coverage for infants under 36 months of age was 96–97% in 2017 [4]. Furthermore, the national immunization program budget is approximately 250 million dollars [5].

Despite these efforts, pertussis outbreaks occur every 3 years in South Korea and has recently increased [6]. Therefore, it is evident that the pertussis outbreak is not properly controlled. The reason for this includes non-immunization in adolescents and adults aged ≥ 15 years in a timely manner and underdiagnosis due to atypical symptoms in adolescents and adults [3, 7]. Thus, such patients are the major cause of transmission within their families.

The gold standard diagnosis for pertussis is culture. Nevertheless, recently, polymerase chain reaction (PCR) is widely used because of the rapid diagnosis rate and high sensitivity. Particlularly, multiplex PCR (mPCR) is faster because different pathogens can be detected in only one trial. Therefore, this test is used for the diagnosis of many infectious disease including respiratory infectious disease [8–10], infectious gastroenteritis [11], and infections of the central nervous system such as meningitis and encephalitis [12]. This study was conducted retrospectively to investigate the efficacy of mPCR for the early diagnosis and treatment of pertussis.

Methods

1. Study population and samples

A retrospective study was conducted using the records of 27 patients diagnosed with pertussis from May 2017 to June 2019 at Yeungnam University Hospital. Nasopharyngeal swabs were obtained from all patients and tested using mPCR. The medical records collected included data on age, sex, symptoms at the time of diagnosis, admission, hospitalization, isolation, vaccination history, past medical history, and accompanying diseases. The collected medical records were used to identify the presenting symptoms of pertussis, initiation of treatment, duration of hospitalization, and time needed for diagnosis.

2. Multiplex PCR

The mPCR was conducted with FilmArray respiratory panel (FA-RP; BioFire Diagnostics, Inc., Salt Lake City, UT, USA), using BioFire FilmArray 2.0 equipment, in accordance with the methods recommended by the manufacturer. The FA-RP utilizes a pouch system and is a disposable hermetic system that provides the chemistry needed to isolate, amplify and detect nucleic acids from multiple respiratory pathogens in a single nasopharyngeal swab sample. The FA-RP is a fully automated nested mPCR test that simultaneously detects adenovirus (AdV), coronavirus (CoV)-229E, CoV-HKU1, CoV-NL63, hCoV-OC43,

human metapneumovirus (hMPV), human rhinovirus/enterovirus (HRV/EV), influenza A(Flu A), influenza A/H1N1 (Flu A/H1N1), influenza A/H1-2009 (Flu A/H1-2009), influenza A/H3N2 (Flu A/H3N2), influenza B (Flu B), parainfluenza virus types 1-4 (PIV 1-4), respiratory syncytial virus (RSV), *bordetella pertussis*, *chlamydophila pneumoniae*, and *mycoplasma pneumoniae*. In cases with coinfections, each of the infections by viruses or bacteria were recognized as positive.

3. Statistical analyses

Statistical analyses were performed using the SPSS 25.0 (IBM Corp., Armonk, N.Y., USA). Pearson's Correlation test was used for analyzing the relationship between age and cough duration. P-value less than 0.05 were considered statistically significant.

Ethics statement

This study was approved by the institutional review boards (IRB) of Yeungnam University Hospital (IRB approval number: YUMC 2019-08-013). Informed consent was waived due to the retrospective nature of this study.

Results

Demographics and clinical symptoms

Twenty-seven patients, with a mean age of 48.9 (range 3.3–82.2) years and a male to female ratio of 1:2, were diagnosed with pertussis using mPCR. (Table 1). There were 8 children and adolescents (0–19.9 years) and 17 adults (over 20 years). Heart disease was the most reported underlying medical history, with 9 (33.3%) and 8 (29.6%) patients reporting hypertension and chronic lung disease, respectively. Sputum expectoration (63.0%), dyspnea (n=12, 44.4%), and fever (n=11, 40.7%) were the most common symptoms, besides cough. Peak incidence of pertussis occurred in November in seven patients, while it occurred in three patients in January, February, and August, respectively (Figure 1A). Based on the seasonal pattern, the number of patients with pertussis peaked at 11 (40.7%) in the fall, followed by 8 (29.6%), 5 (18.5%), and 3 (11.1%) in the winter, summer, and spring, respectively. (Figure 1B)

Laboratory and mPCR results

White blood cell (WBC) count, lymphocyte percentage, hemoglobin, and platelet were 14,193 ± 11,538 /uL; 19.3 ± 10.0%; 12.8 ± 1.3 g/dL; and 282,000 ± 73,000 /uL, respectively. Aspartate transaminase (AST),

alanine transaminase (ALT), lactate dehydrogenase (LDH), total bilirubin, bilirubin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were 33 ± 16 IU/L; 22 ± 16 IU/L; 617 ± 323 IU/L; 0.78 ± 0.41 mg/dL; 0.21 ± 0.18 mg/dL; 36 ± 32 mm/hr; and 6.68 ± 9.25 mg/dL, respectively. Procalcitonin and lactate were 1.841 ± 3.121 ng/dL and 2.6 ± 4.2 mmol/L, respectively.

In mPCR, pathogens coinfected with pertussis were found in 10 patients, and AdV and HRV/EV were the most common organisms with (5, 18.5% each), followed by CoV-NL63 (1, 3.7%) and M. *pneumoniae* (1, 3.7%). The median of turnaround time (TAT) of FA-RP was 94 minutes (57–213 min).

Duration of cough

Median duration of cough was 7 days (2–30 days). Seventeen (62.9%) and 24 (88.8%) patients had a coughing period of <8 days and ≤14 days, respectively. Median time from first symptom to diagnosis was 9.0 (1–31) days. Twenty-four patients (81.5%) were diagnosed within 2 weeks. (Table 2). No significant differences between cough duration and age (p = 0.630).

Treatment & Outcome of patients

Twenty-five of 27 patients and one patient were treated with azithromycin and clarithromycin, respectively. All patients were isolated, with 22 (81.5%) hospitalized, and 5 treated at the outpatient clinic. Three patients requiring ventilator care were treated at the intensive care unit. All the 27 patients survived.

Discussion

Pertussis has an incubation period of 7 to 10 days [13]. The clinical course of pertussis progresses through the catarrhal, paroxysmal, and convalescent stage in sequence. Each stage lasts approximately 1 to 3 weeks. Infants and children, and adolescents and adults have similar progression. However, adults and adolescents have milder symptoms than infants and children. In the catarrhal stage, patients present symptoms of upper respiratory tract infections like low-grade fever, malaise, sore throat, nasal congestion, rhinorrhea, lacrimation, sneezing, and nocturnal cough paroxysms. Therefore, it is easy to overlook pertussis at the early stage. In the paroxysmal stage, patients show severe symptoms with intense and violent cough (5 to 10 coughs/paroxysms) that last several minutes and are associated with cyanosis, eye proptosis, tongue protrusion, salivation, thick oral mucus production, lacrimation, and engorgement of neck veins. Furthermore, the classical sign of pertussis—inspiratory whooping—manifests at this stage. Inspiratory whooping, paroxysmal cough, and post-tussive vomiting are the three classical signs of pertussis (CSP) [13]. In the convalescent stage, patients' symptoms gradually decrease.

The WHO clinical case definition is a coughing illness lasting at least 2 weeks with paroxysms of coughing, inspiratory whooping, or post-tussive vomiting. Diagnostic methods of pertussis include culture, PCR, serologic testing, and direct fluorescent antibody (DFA) staining. Serologic test needs to be calibrated to the reference standard for single time point assays after measuring IgG antibody, such as the WHO International Standard [14]. After vaccination, pertussis could not be diagnosed due to vaccine-induced IgG. DFA staining cannot be used to diagnose due to its low sensitivity and specificity. The gold standard for diagnosis of pertussis is laboratory culture. However, the culture growth of B. *pertussis* can take up to 10 days and also requires antimicrobial susceptibility testing and molecular typing. Thus, PCR, which can be diagnosed in one day and has high specificity and sensitivity, can be used as a complement or can replace culture testing in diagnosing patients with clinical symptoms of pertussis [4].

FARP, a recently developed mPCR test, can detect multiple pathogens at one test. In our recent study, we reported that this test significantly shortened the TAT compared to the conventional mPCR [9]. In this study, a median TAT was 94 minutes, and all patients were isolated and received specific antibiotic therapy after diagnosis of pertussis.

Yoon et al. reported that cough duration in patients with pertussis was an average 26.2 of (10-45) days for pediatric adolescents (0–19 years) during 2005-2017 in South Korea[6]. Park et al. reported a median cough duration of 14.0 days (7–21 days for adolescents and adults [≥11 years]) during 2011–2012 [15]. In our study, the median cough duration was 7 days which was shorter than that in previous studies. The shortened time from symptom onset to diagnosis may be due to the rapid mPCR test.

There was no significant difference in cough duration between pediatric adolescents and adults (Figure 3; p=0.711); the cough duration for patients diagnosed with pertussis was 7 days on median, 8.48 days on average, which was less than that in previous studies [6, 7, 15]. In this study, we retrospectively analyzed the clinical symptoms of patients diagnosed with pertussis. Only 9 patients (33.3%) showed CSP, which seemed to be the result of early diagnosis.

In South Korea, according to the National Vaccination Management Guidelines, all citizens received three basic vaccinations with DTaP at 2, 4 and 6 months of age, three booster vaccinations at 15–18 months, with DTaP at 4 to 6 years of age; with Tdap at 11 to 12 years of age, followed by a dose of Td vaccine every 10 years [16]. In 2017, DTaP immunization rates for babies who were 12 months, 24 months, and 36 months were reported to be 97.7%, 96.2%, and 96.6%, respectively[5, 17]. Despite this high immunization rate, reports on the incidence of pertussis are increasing, and nonetheless, the Korean surveillance system for pertussis is likely to underestimate the burden of pertussis [17, 18].

In addition, most adolescents do not receive regular booster vaccines; it has been reported that the probability of pertussis as a cause of chronic cough has increased [7]. Furthermore, pertussis infection in adolescents and adults has been found to be responsible for household transmission of pertussis to susceptible infants [19].

In the US, reported pertussis incidence has increased since the 1980s, with peaks every 2–5 years. In 2013, there were 28,639 cases of reported pertussis in the US and 13 pertussis-related deaths [20]. In the US, a claims database analysis study showed that considerable underreporting of pertussis in people aged under 50 years exists, especially with increasing age. Thus, it is necessary to develop public health programs to reduce the pertussis burden [21]. In our study, adolescents and adults infected with pertussis accounted for 85.2% of the total burden. Therefore, if the mPCR test using FA-RP is applied to patients in this age group, the outbreak of pertussis may be more effectively controlled.

Conclusions

In conclusion, testing of patients with respiratory symptoms through mPCR containing pertussis can lead to early diagnosis. Early diagnosis and proper treatment, may help in outbreak control of pertussis.

Declarations

Competing interests

The authors declare that they have no competing interests.

List of Abbreviations

AdV; adenovirus

ALT; alanine transaminase

AST; Aspartate transaminase

CSP; classical signs of pertussis

CoV; coronavirus

CRP; C-reactive protein

DFA; direct fluorescent antibody

DTaP; diphtheria, tetanus, and acellular pertussis

ESR; erythrocyte sedimentation rate

FA-RP; FilmArray respiratory panel test

hMPV; human metapneumovirus

HRV/EV; human rhinovirus/enterovirus

Flu A; influenza A

Flu A/H1N1; influenza A/H1N1

Flu A/H1-2009; influenza A/H1-2009

Flu A/H3N2; influenza A/H3N2

Flu B; influenza B

LDH; lactate dehydrogenase

mPCR; multiplex PCR

NIP; National Immunization Program

PIV 1-4; parainfluenza virus types 1-4

PCR; polymerase chain reaction

RSV; respiratory syncytial virus

TAT; turnaround time

WBC; White blood cell count

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Author's contribution

JML and JYA is the co-principal investigator for this study. JML, JHL, and YKK conceived the idea for this manuscript. SCO and SMP carried out the data analysis and wrote the first draft of the manuscript. SCO, SMP, JH, EYC, HJJ, YKK, JHL, JYA and JML interpreted the results and critically reviewed drafts of this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The research proposal was reviewed and approved by the Ethical Review Board of Yeungnam University Hospital (IRB approval number: YUMC 2019-08-013) before executing the study. The study complies with local guidelines that state consent is not needed for this type of study.

Consent for publication

Not applicable.

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Tables

Table 1. Characteristics of patients

Characteristics		N (%)
Number of patients		27
Age, median (range)		48.9 (3.3–82.2)
Male		9 (33.3)
Underlying medical history		
	Diabetes	4 (14.8)
	Hypertension	9 (33.3)
	Chronic lung disease	8 (29.6)
	Asthma	6 (22.2)
	COPD	2 (7.4)
	Interstitial lung disease	2 (7.4)
	Heart disease	10 (37.0)
Presenting symptom		
	Fever	11 (40.7)
	Chills	5 (18.5)
	Cough	27 (100.0)
	Sputum	17 (63.0)
	Rhinorrhea	7 (25.9)
	Dyspnea	12 (44.4)
	Post-tussive vomiting	2 (7.4)
	Paroxysmal cough	3 (11.1)
	Inspiratory whooping	9 (33.3)
Laboratory finding (mean ± SD)		
	WBC (/uL)	14193 ± 11538
	Lymph (%)	19.3 ± 10.0
	Hb (g/dL)	12.8 ± 1.3
	Platelet (/uL)	282,000 ± 73,000
	AST (U/L)	33 ± 16
	ALT (U/L)	22 ± 16
	LDH (U/L)	617 ± 323
	TB (mg/dL)	0.78 ± 0.41
	DB (mg/dL)	0.21 ± 0.18
	ESR (mm/hr)	36 ± 32
	CRP (mg/dL)	6.68 ± 9.25

Procalcitonin (ng/dL)	1.841 ± 3.121
Lactate (mmol/L)	2.6 ± 4.2

Table 2. Parameters related to the time to diagnose pertussis

Turnaround time (min)	94.0 (57.0~213.0)
Cough duration (days), median (range)	7 (2-30)
≤ 8 days	17 (63.0)
8-13 days	4 (14.8)
≥ 13 days	4 (14.8)
unknown	2 (7.4)
Time from first symptom to diagnosis (days), median (range)	9.0 (1 - 31)
≤ 8 days	7 (25.9)
8-13 days	15 (55.6)
≥ 13 days	5 (18.5)

Figures

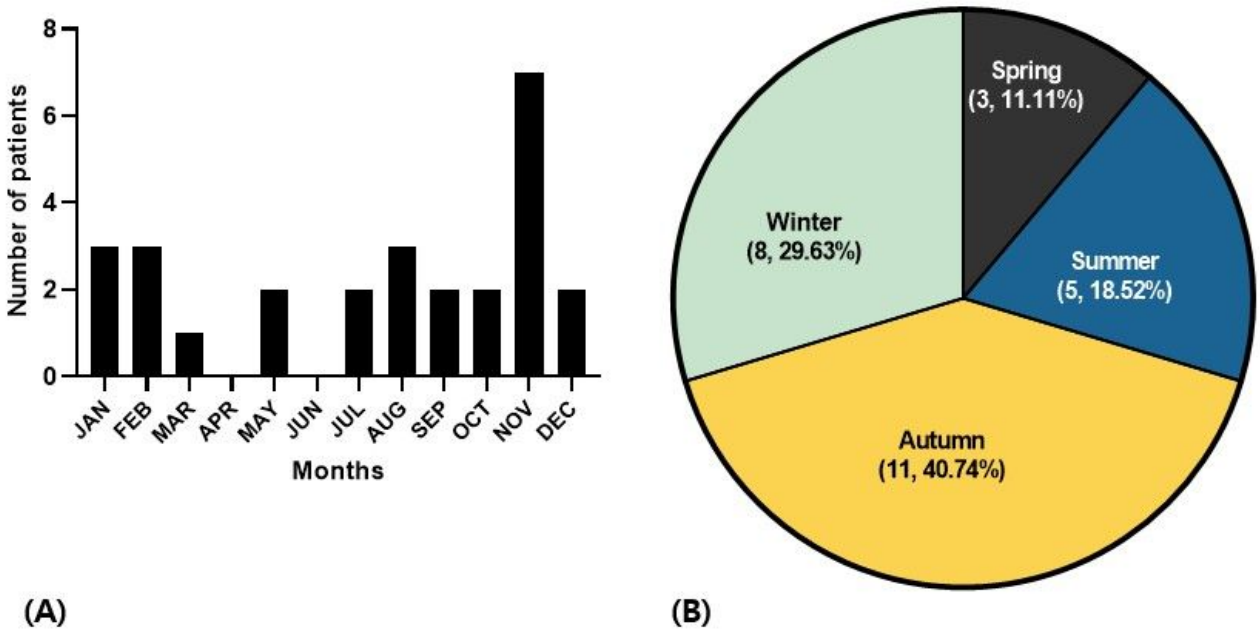

Figure 1

Monthly and seasonal trend of pertussis incidence (A) Monthly trend of pertussis incidence (B) Seasonal trend of pertussis incidence

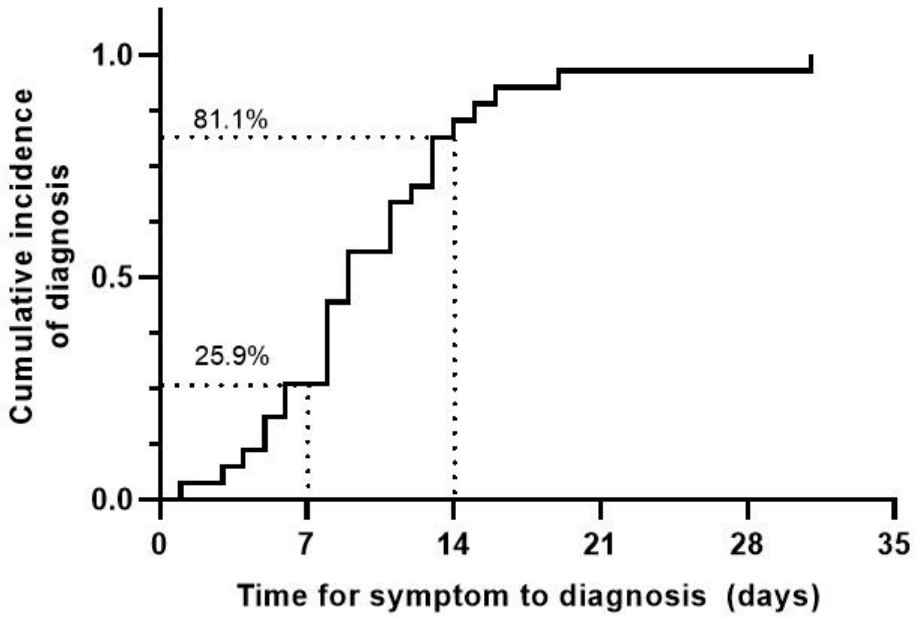

Figure 2

Cumulative incidence for time to diagnosis

Supplementary Files

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- SupplementFigure1.pptx
- Supplementtable1.docx