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The Contribution of Syndromic Diagnostic Tests to Pertussis Epidemiology

Sendromik Tanı Testlerinin Boğmaca Epidemiyolojisine Katkısı

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Abstract

Objective: Pertussis is a vaccine-preventable, contagious respiratory infection. It can cause serious morbidity and mortality especially in young children. In this study, we aimed to investigate the effect of syndromic polymerase chain reaction (PCR) in the diagnosis of pertussis and the clinical features of the patients.

Material and Methods: Nasopharyngeal swab specimens of patients who presented with pertussis-like illness between April and December 2017 were sent to Public Health Microbiology Reference Laboratory for Bordetella pertussis culture and PCR. Between April and December 2018, nasopharyngeal swab specimens were tested with the FilmArray® Multiplex PCR (BioFire, Biomerieux Diagnostics, France) in Microbiology Laboratory of our hospital The number of patients diagnosed with pertussis was compared between April-December 2017 and April-December 2018. The clinical features of patients were examined. Nasopharyngeal swab specimens of 7 patients between April and December 2017 were sent to Public Health Microbiology Reference Laboratory. *B. pertussis* culture and PCR positivity were detected in two patients in this period. Between April and December 2018, nasopharyngeal swab specimens of 17 patients were found to be positive for *B. pertussis* with FilmArray® Multiplex PCR (BioFire, Biomerieux Diagnostics, France). In 2017, there were two definite pertussis cases. With the introduction of the syndromic PCR in 2018, all 17 patients were recorded as definite pertussis cases.

Results: Between April and December 2018, of all patients with *B. pertussis* infections, 10 (59%) were female, 7 (41%) were male. The mean age was 1.9 months (range, 1 to 3 months). 59% (n= 10) of cases were unvaccinated and 41% (n= 7) had one dose of pertussis vaccine. The cocoon strategy was implemented to none of the parents. The mean time between sending nasopharyngeal swab samples and obtaining results was 2.7 (range, 1

Öz

Giriş: Boğmaca, aşı ile önlenebilir, bulaşıcı bir solunum yolu enfeksiyonudur. Tüm yaş gruplarını etkilemekle birlikte, özellikle küçük çocuklarda ciddi morbidite ve mortaliteye neden olabilir. Bu çalışmada, boğmaca tanısında sendromik polimeraz zincir reaksiyonunun (PCR) etkisinin ve hastaların klinik özelliklerinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Nisan-Aralık 2017 tarihleri arasında boğmaca benzeri klinik tablo ile hastanemize başvuran hastaların nazofarenks sürüntü örnekleri *Bordetella pertussis*'in kültür ve PCR ile araştırılması için Halk Sağlığı Genel Müdürlüğü Mikrobiyoloji Referans Laboratuvarına gönderildi. Nisan-Aralık 2018 tarihleri arasında boğmaca benzeri klinik tablo ile hastanemize başvuran hastaların nazofarenks sürüntü örneklerinde hastanemiz Mikrobiyoloji Labaratuvarında FilmArray® Multipleks PCR (BioFire, Biomerieux Diagnostics, France) ile *B. pertussis* araştırıldı. Her iki dönemde boğmaca kesin tanısı alan hastaların sayısı karşılaştırıldı, 2018 yılında boğmaca tanısı alan hastaların klinik özellikleri incelendi. Nisan-Aralık 2017 tarihleri arasında nazofarenks sürüntü örneği Halk Sağlığı Genel Müdürlüğü Mikrobiyoloji Referans Laboratuvarına gönderilen 7 hastanın ikisinde hem *B. pertussis* üremesi hem de PCR pozitifliği saptandı. Nisan-Aralık 2018 tarihleri arasında 17 hastada nazofarenks sürüntü örneğinde hastanemiz Mikrobiyoloji Labaratuvarında FilmArray® Multipleks PCR (BioFire, Biomerieux Diagnostics, France) ile *B. pertussis* pozitif saptandı. 2017'de 7 şüpheli boğmaca tanısı olan hastanın ikisi kesin boğmaca vakası olarak kayıtlara geçerken; 2018'de sendromik PCR testinin kullanılmaya başlanmasıyla, 17 hastanın tümü kesin boğmaca vakası olarak kayıtlara geçti.

Bulgular: Nisan-Aralık 2018 tarihleri arasında boğmaca tanısı ile izlenen hastaların 10'u (%59) kız, 7'si (%41) erkek olarak tespit edilmiştir. En küçük hasta 30 günlük, en büyük hasta 3 aylık (ortalama 1.9 ay) idi. Olgula-

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to 6) hours. The mean length of hospitalization was 5.6 (range, 3 to 11) days. One case (6%) required non-invasive mechanical ventilation.

Conclusion: Pertussis may cause severe clinical conditions especially in infants under 6 months. Rapid diagnosis of pertussis with syndromic PCR makes a significant contribution to pertussis epidemiology. It also improves the timely diagnosis, postexposure prophylaxis and management.

Keywords: Pertussis, syndromic polymerase chain reaction, child, epidemiology

rın %59'u (n= 10) aşısızdı, % 41'ine (n= 7) tek doz aşı uygulanmıştı. Hiçbir hastaya koza stratejisi uygulanmamıştı. Nazofarens sürüntü örneklerinin laboratuvara gönderilmesi ve sonuç elde edilmesi arasındaki ortalama süre 2.7 (minimum-maksimum: 1-6) saat idi. Ortalama hastanede yatış süresi 5.6 (minimum-maksimum= 3-11) gündü. Bir olgu non-invaziv mekanik ventilatör desteği aldı.

Sonuç: Boğmaca özellikle aşıları tamamlanmamış 6 aydan küçük bebeklerde ağır klinik tablolara neden olabilmektedir. Bu yaş grubunda sendromik PCR ile hızlı tanı konması hastalığın doğru yönetilmesine önemli katkı sağlamaktadır.

Anahtar Kelimeler: Boğmaca, sendromik polimeraz zincir reaksiyonu, çocuk, epidemiyoloji

Introduction

Pertussis is a highly contagious acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis*. Although it may affect all susceptible age groups, it can lead to severe clinical manifestations, hospitalisations and even death in infants and children who have not yet been vaccinated. Humans are the only host for B.pertussis, and transmission to other individuals occurs via airborne droplets. Adolescents and adults constitute an important source of disease transmission to infants aged younger than 6 months who have not yet been immunised or completely vaccinated (1-4). Despite high vaccination rates, pertussis continues to be an infectious disease that affects all age groups, especially adolescents and infants aged younger than 6 months (5). Early diagnosis and treatment of pertussis is important to reduce morbidity and mortality as well as to prevent transmission of the disease to susceptible individuals. Although culture has been recognised as the gold standard method for diagnosis, its sensitivity is low and it may take a long time to yield a result. Therefore, molecular methods providing rapid results with high sensitivity and specificity have been developed to promptly identify the responsible microorganisms and initiate appropriate therapy in a timely manner (6-8). This study aimed to investigate the effect of syndromic polymerase chain reaction (PCR) on the diagnosis of pertussis and its clinical manifestation in patients.

Materials and Methods

This study was conducted with the approval of the Clinical Research Ethics Committee of our hospital (Decision no: 09.2019.346 Date: 05.04.2019).

Nasopharyngeal swabs of the patients who were admitted to our hospital with pertussis-like clinical manifestation between April and December 2017 were evaluated at the Microbiology Reference Laboratory of Public Health for culture and PCR testing for *B. pertussis*. Nasopharyngeal swabs of the patients who were admitted to our hospital with pertussis-like clinical manifestations between April and December 2017 were tested for *B. pertussis* at our hospital's microbiology laboratory using FilmArray® Multipleks PCR (BioFire, Biomerieux Diagnostics, France).

Nasopharyngeal swabs obtained from the patients using eSwab (Flocked swab, Copan Diagnostics, Italy) were sent to the microbiology laboratory of our hospital in a viral transport medium. From the samples, nucleic acids of 17 viruses [adenovirus (AdV), coronavirus (CoV) HKU1, CoV NL63, CoV 229E, CoV OC43, human metapneumovirus (hMPN), human rhinovirus/enterovirus (hRV/Entero), influenza A,A/H1, A/H3, A/H1-2009 ve B, parainfluenza (PIV) 1, 2, 3 and 4, respiratory syncytial virus] and three bacteria (*B. pertussis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*) were tested by a multiplex nested PCR based test (FilmArray Respiratory Panel, Biomerieux Diagnostics, France) on Biofire, FilmArray 2.0 system.

The DNA and RNA process controllers are evaluated first when evaluating the test results. Both controls must work to ensure test validity. Subsequently, the melting curve analyses conducted in three separate wells for each agent are evaluated. If the three melting curve analyses are positive and if the difference between the Tm of each agent is less than 1°C, the report is issued as 'detected'.

Statistical analyses were conducted using SPSS version 16.0 software (IBM, USA, 2009). Mean values were used for continuous variables in the presentation of clinical findings. Frequency and percentages were used to summarise categorical data.

Results

Nasopharyngeal swabs of seven patients who were admitted to the Pediatric Emergency Department of our hospital and hospitalised at the Pediatric Infectious Diseases Clinic with pertussis-like clinical manifestation between April and December 2017 were sent to the Microbiology Reference Laboratory of Public Health. Culture and PCR positivity was detected for *B. pertussis* in two patients. Nasopharyngeal swabs of 17 patients who were admitted to the same centre with pertussis-like clinical manifestation between April and December 2018 tested positive for *B. pertussis* by Film Array Multiplex PCR at our hospital's microbiology laboratory. In 2017,

two of seven patients with suspected pertussis were classified as definitive pertussis cases, whereas in 2018, when syndromic PCR testing became available all 17 patients were classified as definitive pertussis cases.

Of the patients followed up with a diagnosis of pertussis between April and December 2018, 10 (59%) were females and 7 (41%) were males. The youngest patient was aged 30 days and the oldest patient was aged three months, with mean age being 1.9 months. Of these cases, 59% (n= 10) were unvaccinated and 41% (n= 7) had received a single dose of vaccine. Cocoon strategy was not applied in any of the patients. All patients had whooping cough, five (29%) had vomiting, and none had fever. Three patients (17.5%) had rales in the lungs and six (35%) had a decrease in breastfeeding frequency. Mean white blood cell count at the time of admission was 19.747 (range, 9800-39300) /mm^3. Mean duration of symptoms was 6.2 days (range, 1-15) days. Nine (53%) of the patients had at least one viral pathogen that concomitantly occurred with *B. pertussis*. Of patients with concomitant viral pathogens, 6 (35%) had rhinovirus/enterovirus, one (6%) had coronavirus NL 63, one (6%) had rhinovirus/enterovirus and parainfluenza virus type 3 and one (6%) had rhinovirus/enterovirus and coronavirus NL 63. The demographic and clinical characteristics of the patients are summarised in Table 1.

Mean time between delivery of nasopharyngeal swabs to the laboratory to be tested by PCR and the results was 2.7 (range, 1-6) hours.

Table 1. General and clinical characteristics of the patient

Characteristics	Number of the patient (frequency %)
Age	
1-2 month	10 (59%)
2-3 month	7 (41%)
Sex	
Female	10 (59%)
Male	7 (41%)
Clinical symptoms	
Paroxysmal cough	17 (100%)
Vomiting	5 (29%)
Pertussis vaccination	
Unvaccinated	10 (59%)
Single dose	7 (41%)
Patient with concomitant viral pathogens	9 (53%)
Concomitant viral pathogens	
Rhinovirus/enterovirus	6 (35%)
Coronavirus NL 63	1 (6%)
Rhinovirus/enterovirus and parainfluenza virus type 3	1 (6%)
Rhinovirus/enterovirus and coronavirus NL 63	1 (6%)
Treatment	
Clarithromycin	6 (35%)
Azithromycin	11 (65%)

Mean time from the onset of clinical symptoms to diagnosis was 6.2 (range, 1-15) days. Three (17.5%) patients had cough for more than 14 days. Mean duration of hospitalisation was 5.6 (range, 3-11) days. No patient had a history of antibiotic use prior to admission. Six of the patients were treated with clarithromycin for 10 days, and 11 were treated with azithromycin for five days. One patient (6%) received non-invasive mechanical ventilatory support. One patient received ampicillin and cefotaxime in addition to clarithromycin. All patients were discharged without any complication and sequelae.

Discussion

Pertussis continues to be a public health issue even in developed countries, where high vaccination rates and adherence to vaccination programmes are observed (9,10). This can be explained by the decrease in the number of antibodies induced by pertussis infection or pertussis vaccine, genetic alterations in *B. pertussis* and improved recognition of and an increase in reporting rates for pertussis cases with the introduction of new diagnostic laboratory tests (11). Typical pertussis symptoms, such as paroxysmal cough, inspiratory stridor and post-coughing vomiting are seen less frequently in older children, leading to delayed diagnosis and prolonged transmission of the disease to other individuals (12). Although the clinical manifestation of pertussis varies according to age and vaccination status, the World Health Organization and Centres for Disease Control and Prevention (CDC) have established the case definition for pertussis. However, microbiological and clinical diagnosis of *B. pertussis* infection is challenging. Microbiological diagnosis is established by culture, antigen detection, PCR and serological methods, with conventional culture being the gold standard method. However, microbiological tests may show false negative results because of the absence of viable microorganisms if the patient is in the late periods of infection and have received antibiotic treatment. Although culture has been considered to be the most specific method to diagnose *B. pertussis* infection, it has low sensitivity and does not provide rapid results. The sensitivity of the culture may vary depending on factors such as time from symptom onset, age, vaccination status, use of antibiotics prior to admission, sampling method, time of delivery to the laboratory and the culture medium used (13). The rate of isolation of the agent is higher in the first two weeks after the onset of disease symptoms. Various studies in the literature have reported a culture sensitivity in the range of 12%-60% (14).

Nucleic acid amplification tests used in molecular diagnosis of *B. pertussis* infection are more advantageous because of their high specificity and sensitivity and because they provide rapid results by detecting microorganisms within hours, are able to detect the agent even in the absence limited presence of viable bacteria and are less affected by antibiotic treatment (15). Owing to these advantages compared with the culture

method, nucleic acid amplification tests are currently more widely used for the diagnosis of pertussis. Factors affecting PCR sensitivity include sample quality, disease stage and duration, use of antibiotics during this period and amplification conditions and their efficacies (8). Similar to that in the culture test, the sensitivity of PCR decreases with increasing time after the onset of clinical symptoms. Unlike culture tests; however, PCR can detect *B. pertussis* in nasopharyngeal swabs after four weeks as the method does not depend on the presence of viable microorganisms. In our study, only three (17.5%) patients had cough for more than 14 days and none had a history of antibiotic use prior to admission.

In the literature, several studies have compared culture vs. PCR methods for the diagnosis of pertussis. In a study by Gürsel et al. (16), the culture of nasopharyngeal swabs of 51 patients showed *B. pertussis* growth and the culture of nasopharyngeal swabs of six (11.8%) tested positive for *B. pertussis* by IS481 Rt-PCR. A study by Dragsted et al. (17) has reported higher sensitivity of IS481 PCR (93%) compared with that of the culture method (58%). A study by Lee et al. (18) evaluating culture vs. PCR vs. serological methods for the diagnosis of *B. pertussis* has reported sensitivities of 64% for culture and 90.6% for PCR.

Pertussis affects all age groups but occurs more commonly and exhibits a more severe disease course in infants younger than three months who have not yet started a vaccination programme or have completed the vaccination schedule. In our study, all patients were aged younger than three months. Ten patients who were followed up with pertussis at our clinic had not received DaBT vaccine as they were aged younger than two months. The remaining seven patients had received a single dose of vaccine, as their age ranged from two to three months. A cross-sectional study by El Basha et al. (19) investigating multiplex PCR and atypical agents in community-acquired pneumonia in 400 children has reported that all children diagnosed with *B. pertussis* infection were aged younger than four months and were younger than those infected with other agents of atypical pneumonia and who had not completed the vaccination schedule. All patients were less than three months of age when vaccination against pertussis had not been initiated or completed and the caregivers who were in close contact with the infants were unvaccinated, which shows the importance of the cocoon strategy. The cocoon strategy aims to prevent the transmission of infectious agents by provision of a vaccination service to all individuals who are in close contact with infants to protect the infants against pertussis, particularly within the first six months of life, during which the level of protective antibodies transferred from mother to infant gradually decreases (20-22). In the literature, it has been reported that the main source of infection for infants who are at a high risk for pertussis is the family members who are close contact with them (23-25). A study by Del Valle-Mendoza et al. (26) conducted on children less than five years of age has found that the most common symptomatic source was the mother (27.8%), followed by an uncle and aunt (22.9%) in patients diagnosed with pertussis. A study by Skoff et al. (24) involving 1.306 infants diagnosed with pertussis has reported that the source of infection in infants less than one year of age was the family members in more than 66%, of which 35.5% were the siblings, 20.6% were the mothers and 10% were the fathers. In a study conducted in Turkey, it has been found that the source of infection was mothers in 42.8% of infants under one year of age who were diagnosed with pertussis (27). As seen in previous studies and in our study, since the age group most vulnerable to pertussis infection comprises infants less than six months of age, the family members who are in close contact with them need to be immunised to protect this age group against pertussis. For this purpose, administration of Tdap vaccine during pregnancy and including pertussis booster doses in the national vaccination schedule for pre-school children and adolescents can be considered.

Paroxysmal cough, which was seen in all patients in our study, was a common symptom observed in patients with pertussis in previous studies, while fever, which has not been included in the case definition for pertussis and which often does not occur during the disease course, was not detected in any of our patients (2,26). In accordance with the literature, the mean white blood cell count was found to be high.

In 2017, mean time from delivery of nasopharyngeal swabs to the Microbiology Reference Laboratory of Public Health for culture and PCR testing and receipt of the results was 14 days. In 2018, the mean time from delivery of nasopharyngeal swabs to the hospital's laboratory for PCR testing to receipt of the results decreased to 2.7 hours with the introduction of multiplex PCR. Li et al. (28) have reported that the pathogen of respiratory tract infections is detected within 65 minutes using the FilmArray airway panel. In our study, we think that the rapid diagnosis of children with suspected pertussis by the PCR method and the timely initiation of appropriate treatment contributed to the successful treatment of all patients without any complications. In addition to the timely initiation of appropriate treatment for the patients, the administration of pertussis prophylaxis to individuals who are in close contact with them is also useful in preventing the transmission of infection.

In conclusion, it is useful to use syndromic PCR tests more widely because of the atypical clinical signs of pertussis in both young children and adolescents, low sensitivity of other methods used in microbiological diagnosis and the long time to obtain results. Thus, reducing the mortality and morbidity by early diagnosis and reducing the contagious period by providing prophylaxis to individuals who are in close contact might contribute to epidemiological studies.

Ethics Committe Approval: The approval for this study was obtained from Marmara University Clinical Research Ethics Committee. (Date: 05.04.2019 Decision no: 09.2019.346).

Informed Consent: Patient consent was obtained.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - NY, EKK; Design - NY, EKK; Supervision - NY, RCS; Resource - RCS, GS; Data Collection and/or Processing - NY, RCS; Analysis and/or Interpretation - NY, EKK, RCS, GS; Literature Search - NY, EKK; Writing - NY, EKK, RCS, GS; Critical Review - EKK, GS.

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