RESEARCH ARTICLE | JANUARY 10 2019

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AIP Conf. Proc. 2058, 020035 (2019)
https://doi.org/10.1063/1.5085548

Study On Pulmonary Function Changes in Infants with Human Bocavirus or Mycoplasma Pneumonia

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Key words: Pulmonary Function; Human Bocavirus; Mycoplasma Pneumonia.

INTRODUCTION

Studies have confirmed that newfound human Bokavirus (HBoV) is an important pathogen of lower respiratory tract infection, and it is closely related to the occurrence of wheezing in infants and children [1, 2]. While its respiration mechanism and the impact of pulmonary function after infection has not yet been finalized. Mycoplasma pneumonia (MP) is also the pathogen of lower respiratory tract infection which is closely associated with whezzing. It has been studied that the types of pulmonary function damage of mycoplasma pneumoniae pneumonia (MPP) is mainly the small airway dysfunction [3]. Lung function can objectively reflect the pathophysiological changes of respiratory system diseases and the extent of their lesions. In the past, pulmonary function test was mainly performed to test the indexes in the process of forced respiration, which required good cooperation of patients, but limited the application of pulmonary function test in infants to some extent. Therefore, the pulmonary function test is only limited to adults or elder children. In recent 10 years, the application of tidal breathing lung function detection technology in children, such as the tibal breathing flow-volume loops (TBFVLs) and various parameters, makes the study of the pulmonary function in children, especially infants become possible. This study have compared the pulmonary function changes in infants with human bocavirus (HBoV) or mycoplasma pneumonia (MP). The aim of this study is to explore the pulmonary function changes in infants with HBoV pneumonia and MP pneumonia and the pathogenic mechanism of HBoV after lower respiratory tract infection. And the purpose of this study is to invesgate the effect of pathologic damage of HBoV lower respiratory tract infection on pulmonary function, and to provide an objective clinical index for the treatment and prognosis evaluation.

MATERIALS AND METHODS

Objects

140 infants who were admitted to our hospital due to bronchial pneumonia from January 2013 to October 2013 were selected, and the sputum pathogen detection results showed that HBoV-DNA was detected in 64 ceses and MP-

International Conference on Frontiers of Biological Sciences and Engineering (FBSE 2018)
AIP Conf. Proc. 2058, 020035-1–020035-4; https://doi.org/10.1063/1.5085548
Published by AIP Publishing. 978-0-7354-1786-1/$30.00

DNA 76 cases. The diagnostic criteria of pneumonia was in accordance with the seventh edition of "practical paidonosology". The main clinical manifestations were cough combined with fever, wheezing, shortness of breath, the moist rales or wheezing rales heard in lungs, and the spot film or patchy oozing of shadows seen in chest x-ray film.

Grouping

The pneumonia patients after admission to hospital who were diagnosed as HBoV and MP infection through sputum pathogen detection were divided into the following groups:

(1) HBoV pneumonia group: 64 cases. According to age: < 1 year old group: 31 cases, weight (8.63±1.80) kg; 1-3 years old group: 33 cases, weight (11.97±3.16) kg.

(2) MP pneumonia group: 76 cases. According to age: < 1 year old group: 31 cases, weight (8.43±2.54) kg; 1-3 years old group: 45 cases, weight (12.88±2.45) kg.

(3) Normal control group: 38 cases who had received health examination in our hospital, including 19 male cases and 19 female cases. According to age: < 1 year old group: 15 cases, weight (7.87±2.50) kg; 1-3 years old group: 23 cases, weight (14.76±2.35) kg.

Inclusion criteria: no abnormalities in physical examination; no respiratory infection in the past 1 month; no congenital heart disease; no history of asthma in the family; no upper respiratory tract infection in the last two weeks; no other diseases lead to respiratory central nervous system disorders; no congenital laryngeal cartilage hypoplasia; no lung malformations.

Methods

Specimen Collection

Within 24 h after admission to the hospital, a disposable suction tube was put into the nasal of patients about 7-9 cm to drew sputum 1-2 ml under the negative pressure, and then submitted them for inspection within half an hour after diluted with 3 ml sterile saline solution.

Detection of HBoV by Real Time Fluorescence Quantitative PCR

Sputum sample was washed with PH=7.0 PBS, and to took the sediment to join DNA lysate, and to collect the supernatant after centrifugation; HBoV PCR reaction solution A 17 ul and HBoV PCR reaction solution B 3 ul were added respectively to the PCR reaction tube; The above PCR tubes were added with the processed negative quality control, the nuclear acid samples to be tested, the HBoV positive quality control with 5 ul respectively, and to centrifuge them under 8000 rpm for several seconds, and to add real-time fluorescent PCR automatic cycler (BIO-RAD icycle company): 50°C 2 min, 1 cycle; 90°C 15 min, 1 cycle; 94°C 15 s-55°C 45 s (collecting fluorescent), 40 cycles. HBoV PCR reaction solution A1, HBoV PCR reaction solution B, negative quality control, HBoV positive quality control were all produced by Daan Gene Co., Ltd. of Sun Yat-sen University. The amplification curve in FAM detection channel had a logarithmic growth period and the Ct value ≤ 36 was positive results. The fluorescence quantitative PCR instrument was Light Cycler 480. The specific operation was completed by the professional technicians of Department of Pediatrics, Children's Hospital Affiliated to Suzhou University.

Detection of MP by Real Time Fluorescence Quantitative PCR

4 times normal saline was added in sputum, and put it in the 4°C refrigerator through overnight after blow and beat by a pipette so as to make sputum fully liquified; the mixture liquid was centeifuged for 5 min in the centrifuge tube at 12000 rpm, and the precipitate was added into the DNA to get the mixture, and then the supernatant was fetched after centrifugation; MP PCR reaction solution and Taq enzyme were added in the PCR reaction tube. The above PCR tube were added to the 2 ul treated supernatant sample, to centrifuge them for several seconds at 8000 rpm, and then to add real-time fluorescent PCR automatic cycler (BIO-RAD icycle company): 93°C 2 min, 93°C 45 s→55°C 60 s→10 cycles, 93°C 30s→55°C,45s→30 cycles. MP PCR reaction solution, Taq enzyme, negative quality control and MP positive quality control were all produced by Daan Gene Co., Ltd of Sun Yat-sen University. The amplification curve in FAM detection channel had a logarithmic growth period and the Ct value ≤ 36 was positive results. The

fluorescence quantitative PCR instrument was Light Cycler 480. The specific operation was completed by the professional technicians of Department of Pediatrics, Children's Hospital Affiliated to Suzhou University.

Tidal Breathing Lung Function Detection

Within 2 days after admission to the hospital and 2 h after eating, respiratory secretions of subjects were removed at first so as to maintain airway patency. The tidal breathing pulmonary function indicators were monitored at a supine position under the natural sleep condition. A soft, resilient mask attached to the current meter was placed into the mouth and nose of the subjects with moderately pressure to avoid gas leakage. The flow rate and capacity of pulmonary function should be corrected regularly (1 time per day). About 10-30 tidal breath flow-volume loops (TBFVLs) were tested each time, and the computer automatically selected the appropriate TBFVLs (not less than 10 loops), then the mean and standard deviation of each parameter was calculated. The instrument was the Master Scope pulmonary function from Jaeger, German, and was operated by specially-assigned person.

Detection index: respiratory rate (RR), tidal volume per kilogram (TV/kg), peak tidal expiratory flow (PTEF), peak time of tidal expiratory flow (TPTEF), peak velocity of tidal expiratory flow (VPTEF), peak velocity of tidal expiratory flow/expiratory volume (VPTEF/VE), the tidal expiratory flow at 25% of the remaining tidal volume (TEF25%), the tidal expiratory flow at 25% of the remaining tidal volume / peak flow (25/PF), mean expiratory flow rate / mean inspiratory flow rate (ME/MI).

Statistical Treatment

Pulmonary function instrument was used to automatically calculate the mean values of various parameters, and expressed with $\bar{x}\pm S$. SPASS 17 statistical software was used for statistical processing of the data. The normal distribution of the data was determined by the normal test. The statistical method of normal distribution data was analyzed by the multi-samples Mean-Variance analysis. The LSD test was used to compare between multi group: non-parametric test was used for the statistical method of non-normal distribution data, and $P<0.05$ meant that the difference was statistical significance.

RESULTS

Analysis of Pulmonary Function Indicators in Infants in Hbov and MP Pneumonia Group

In <1 year old group, the TPTEF, VPTEF and small airway function indicators TPTEF/TE, VPTEF/VE, TEF25% and 25/PF of infants with HBoV and MP pneumonia were all lower than that in normal control gorup, and the RR frequency increased, with statistical significance ($P<0.05$), while there was no significant difference of major airway function indicators ME/MI. As shown in Table 1:

TABLE 1. The comparison of various pulmonary function indicators in within 1-year-old infants with HBoV and MP pneumonia ($\bar{x}\pm S$)

	HBoV infection group	MP infection group	Normal control gorup
RR (per time/min)	33.95±10.24*	35.73±11.27*	28.16±2.53
TV/kg (ml/kg)	7.78±1.79	7.39±2.25	8.03±1.90
PTEF (ml/s)	110±51.08	98.39±29.77	112.4±67.17
TPTEF (s)	0.28±0.21*	0.26±0.15*	0.34±0.17
VPTEF (ml)	20.02±11.27*	18.75±12.25*	24.40±18.75
TPTEF/TE (%)	26.07±15.28*	25.86±13.98*	43.56±14.19
VPTEF/VE (%)	29.39±12.54*	28.99±11.60*	42.87±12.84
TEF 25% (ml/s)	66.23±34.98*	60.23±20.50*	90.80±58.67
25/PF (%)	62.69±16.32*	62.19±15.94*	80.33±10.73
ME/MI (%)	78.76±26.48	80.69±38.08	80.18±24.46

Note: Compared with control gorup, *$P<0.05$

In 1-3 years old group, the PTEF, TPTEF, VPTEF, TPTEF/TE, VPTEF/VE, TEF25% and 25/PF of infants with HBoV and MP pneumonia were all significantly lower than that in control group, with statistical significance ($P<0.05$),

and no difference of RR compared with control group. While there was no significant difference about the above indicators between the HBoV pneumonia group and the MP pneumonia group. As shown in Table 2:

TABLE 2. The comparison of various pulmonary function indicators in 1-3 years old infants with HBoV and MP pneumonia ($\bar{x}\pm S$)

	HBoV infection group	MP infection group	Normal control gorup
RR (per time/min)	27.05±4.87	29.54±8.03	25.55±2.66
TV/kg (ml/kg)	8.96±2.32	8.49±2.55	9.55±2.92
PTEF (ml/s)	131.48±47.46*	130.44±43.16*	18187±53.29
TPTEF (s)	0.33±0.16*	0.32±0.15*	0.55±0.26
VPTEF (ml)	30.09±17.53*	31.05±16.12*	63.34±27.48
TPTEF/TE (%)	25.41±13.56*	25.74±11.79*	46.29±10.76
VPTEF/VE (%)	28.02±11.05*	28.56±9.62*	45.05±12.15
TEF 25% (ml/s)	82.39±33.30*	82.20±38.74*	149.30±53.75
25/PF (%)	62.76±17.95*	61.93±20.99*	83.13±4.96
ME/MI (%)	71.02±25.54	66.03±16.79	67.04±10.48

Note: Compared with control gorup, *P<0.05

Morphological Analysis of Tbfvls in Infants with Hbov and MP Pneumonia

The TBFVLs of healthy infants was elliptical. Directly compared from the TBFVLs, the curve shape of HBoV pneumonia, MP pneumonia and normal control group were all changed and characterized by left-shifted PTEF and trough-like concave in descending limb. The kurtosis of 1-3 years old infants with pneumonia were slightly decreased.

DISCUSSION

Pulmonary function test is a comparable objective index to judge the degree of respiratory disease, evaluate the curative effect and infer the prognosis [4]. Tibal breathing is a model of lung functin test, with simple operation, without the understanding and cooperation of the subjects, without special breathing movements, which ensures the accuracy of testing and good repeatability. In our country, the tidal breathing lung function of infant has been used for the diagnosis and treatment monitoring of neonatal pneumonia and asthma [5]. With the gradually matured tibal breathing analysis, there have been some studies on the wheezing, asthma and lung function damage of infants in recent years.Therefore, the study of infant lung function can not only predict the occurrence of asthma and asthma, but also can reflect the pathological mechanism of the body to a certain extent.

Tidal breathing lung function was suitable for the severity judgement and prognosis for infants with respiratory disease, without operation and traumatic. The lung function damage of HBoV and MP pneumonia were all shown as small airway obstructive dysfunction, manifested in the intuitive TBFVLs with left-shifted PTEF and trough-like concave in descending limb. From the damage of pulmonary function, it was speculated that there might be similarities between the pathologic process and pathologic damage of HBoV and MP infection.

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