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Original Article

Correlation between nitric oxide content in exhaled breath condensate and the severity of acute respiratory distress syndrome

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Abstract: Nitric oxide (NO) can induce necrosis and detachment of branchial epithelium, aggregate inflammation and induce acute respiratory distress syndrome (ARDS). The detection of NO in exhaled breath condensate (EBC) has not been widely applied in evaluating ARDS condition. This study thus tested NO in EBC of ARDS patients, in order to provide non-invasive method to monitor disease condition of ARDS. ARDS patients in ICU of our hospital were recruited in parallel with healthy controls. NO contents in EBC and serum were measured in both control group and ARDS patients before/after treatment, and between survived and dead patients. APACHE II score, PaO_2/FiO_2, and PaO_2 were also quantified and analyzed for their correlation with NO contents. Correlation between EBC and serum NO contest was also analyzed. ARDS patients had significantly higher EBC/serum NO contents before treatment compared to control. NO levels were remarkably decreased after treatment ($P<0.05$). Survival patients had decreased APACHEII score, and EBC/serum NO, plus elevated PaO_2/FiO_2, and PaO_2 after treatment. Dead patients had elevated APACHEII score, EBC/serum NO with prolonged treatment, plus decreased PaO_2/FiO_2, and PaO_2 ($P<0.05$). EBC/serum NO in ARDS patients were positively correlated with APACHE II score, and negatively correlated with PaO_2/FiO_2, and PaO_2 ($P<0.05$). NO level in EBC was positively correlated with serum level in ARDS patients ($P<0.05$). The detection of EBC NO content can reflect hypoxia and disease condition of ARDS patients, and benefits treatment efficacy evaluation and prognostic judgement. EBC NO can replace serum NO due to satisfactory correlation.

Keywords: Exhaled breath condensate, nitric oxide, acute respiratory distress syndrome, correlation analysis

Introduction

Acute respiratory distress syndrome (ARDS) is one clinical syndrome caused by intra-or extra-pulmonary factors except cardiac reasons, and is the most severe phase and sub-type of acute lung injury (ALI) [1, 2]. ARDS usually manifests as acute disease onset, and featured as rapid respiration with distress and refractory progressive hypoxemia [3]. Routine oxygen inhalation cannot correct such hypoxia, and X-ray frequently shows diffused infiltration of pulmonary alveoli. Nitric oxide (NO) is one small molecule secreted by endothelium, epithelium and inflammatory cells. During ARDS onset, pulmonary inflammatory cells release large amounts of inflammatory factors to enhance synthesis of nitric oxide synthase (NOS) in alveolar macrophage, neutrophil and bronchial epithelium, thus producing abundant NO to release into the pulmonary tissues [4]. High dosage of NO can directly injury DNA, mitochondrial and iron-containing enzyme activity inside air way tissues, and produce NO_2^-/NO_3^- with even higher toxicity by interacting with O_2^- in unique chemical environment of air way ducts [5]. NO_2^-/NO_3^- can further induce lipid peroxidation to detach, denature or even cause necrosis of pulmonary epithelial cells, aggregate inflammation and induce ARDS onset [6]. Moreover, abundantly released NO can manifest as strong cytotoxicity, elevating infiltration of micro-veins, leading to imbalance of pulmonary ventilation/blood flow ratio and worsening of intra-pulmonary bronchial spasm [7].

Currently the pulmonary inflammation and disease condition of ARDS patients are mainly evaluated on samples of serum, induced sputum (IS) and bronchoscopic alveolar lavage fluid

(BALF) [8]. Compared to IS or BALF, exhaled breath condensate (EBC) can monitor inflammatory response inside airways in a real-time, repetitive and dynamic manner, thus having critical roles for evaluating treatment efficacy and predicting disease prognosis [9]. This study thus investigated NO content in EBC of ARDS patients, in order to provide one non-invasive method to monitor pulmonary oxidative stress and inflammation.

Patients and methods

Research objects

A total of 102 ARDS patients who received mechanical ventilation in ICU of our hospital from September 2015 to June 2016 were recruited. All patients received mechanical ventilation via tracheal intubation or tracheotomy. There were 53 males and 49 females, aging between 42 and 73 years (average age = 61.5 ± 15.7 years). All included cases fitted definition and diagnostic criteria of ARDS in 2012 [10]: (1) Existence of risk factors causing ARDS; (2) Within one week of disease onset; (3) Required positive pressure mechanical ventilation; (4) Severe oxygen binding dysfunction, as defined by oxygenation index (PaO_2/FiO_2) ≤300 mmHg (1 mmHg = 0.133 kPa) when inhalation oxygen concentration (FiO_2) = 1.00 and positive end expiratory pressure (PEEP) ≥5 cmH_2O (1 cmH_2O = 0.098 kPa); (5) Chest film showed bilateral pulmonary infiltration that cannot be explained by pulmonary effusion, atelectasis or nodules. Patients were further divided into survival and dead group based on the prognosis 2 weeks after ICU treatment. There were 82 cases in survival group, including 42 males and 40 females, with aging between 45 and 71 years (average age = 59.4 ± 16.2 years). There were 20 cases of dead group (average survival time in ICU = 19.2 ± 5.8 days), including 11 males and 9 females, aging between 41 and 74 years old (average age = 62.4 ± 16.8 years). Another cohort of 22 healthy individuals were recruited as the control group, including 11 males and 11 females, aging between 42 and 70 years (average age = 61.6 ± 14.7 years).

EBC collection and NO assay

All ARDS patients were admitted into ICU. After diagnosis with ARDS, comprehensive treatment plan including anti-inflammation, anti-symptom, and mechanical ventilation were performed besides treating primary disease. EBC samples were collected before treatment, 3 days and 5 days after treatment when patient's vital signs were relatively stable. 15 min before collection, no sputum aspiration using saline was performed. During sample collection, ventilation tube was replaced by dry sterile screwed tube. Heated miniaturization was detached, whilst EcoScreen condense was aligned into the exhalation site of ventilation tube. Each sample collection took about 20 min for obtaining 2~4 mL EBC samples, which were kept at -80°C for further use. EBC from healthy control people was also collected by the static respiration using a one-way valve coupled with nose clip, after cleaning the oral cavity. 2~4 mL EBC samples were collected after 20 min, and were kept at -80°C for further use. 5 mL peripheral venous blood samples were also collected at the same time. Serum was separated and stored at 80°C for further use.

Observation indexes

ELISA kit (Xitang Bio, China) was applied to test NO concentration in EBC and serum samples following the manual instruction. Artery blood samples were collected from ARDS patients in blood-gas analysis. Oxygenation index (PaO_2/FiO_2) and artery oxygen pressure (PaO_2) were tested before treatment, 3 days and 5 days afterwards.

APACHE II score

Acute physiology and chronic health evaluation scoring system (APACHE II) is the most widely applied evaluation system for critical patients in ICU. It is composed of acute physiology score, age score and chronic health status score. The final score is the summation of those three components, with higher score indicating severer conditions (full mark = 71). All physiological indexes utilized the minimal value within 24 h.

Statistical analysis

SPSS 18.0 was used for data processing. Measurement data were presented as mean ± standard deviation (SD). Comparison of multiple groups was carried out by one-way analysis of variance (ANOVA). Between-group-comparison of means was performed using SNK method.

Table 1. EBC and serum NO content between patients and control people

Group	NO in EBC (μmol/L)	NO in serum (μmol/L)
Healthy control	15.65 ± 4.43	18.76 ± 4.52
ARDS, pre-treatment	47.81 ± 6.05[a]	48.45 ± 6.21[a]
ARDS, 1 day after	33.71 ± 3.12[a,b]	34.51 ± 3.89[a,b]
ARDS, 5 days after	28.78 ± 3.32[a,b,c]	27.33 ± 3.73[a,b,c]

Note: [a], P<0.05 compared to control group; [b], P<0.05 comparing between 1 day after and pre-treatment; [c], P<0.05 comparing between 1 day and 5 days after treatment.

Pearson approach was used for correlation analysis. A statistical significance was defined when P<0.05.

Results

Significantly elevated EBC and serum NO contents in ARDS patients

Test results showed significantly higher EBC/serum NO levels in ARDS patients before treatment (P<0.05 compared to healthy control people). One day after treatment, both EBC and serum NO levels were significantly depressed (P<0.05 compared to those before treatment). At 5 days after treatment, EBC and serum NO contents were significantly lower (P<0.05 compared to 1 day-post treatment), but were still higher than health control (P<0.05, **Table 1**).

APACHE II score and NO content of ARDS patients in survival and dead group

Before treatment, APACHE II score, NO contents in EBC and serum had no significant difference between survival and dead groups (P>0.05). In ARDS survival group, APACHE II score 1 day after treatment, NO contents in EBC and serum were all significantly decreased (P<0.05 compared to pre-treatment level). 5-day post-treatment further decreased those levels (P<0.05). In dead patient group, APACHE II score, NO contents in EBC or serum were instead elevated with time elapsed (**Table 2**).

Better oxygenation status of survived ARDS patients than dead group

Before treatment, PaO_2/FiO_2, and PaO_2 had no significant difference between survival and dead patients (P>0.05). In survival ARDS group, PaO_2/FiO_2, and PaO_2 were all significantly elevated 1 day after treatment (P<0.05). The magnitude of elevated oxygenation status was even higher 5 days after treatment (P<0.05). In dead group, PaO_2/FiO_2, and PaO_2 levels were downregulated with time elapsed (**Table 3**).

Correlation between APACHE II score, PaO_2/FiO_2, and PaO_2 levels and NO levels

Pearson correlation analysis showed significantly positive correlation between NO levels in EBC at 1 day and 5 days after surgery and APACHE II score (P<0.05). Positive correlation also existed between serum NO level and APACHE II score before and 5 days after treatment (P<0.05). EBC NO level as negatively correlated with PaO_2/FiO_2, and PaO_2 levels at all time points before/after treatment (P<0.05). Serum NO level was also negatively correlated with PaO_2/FiO_2, and PaO_2 levels at all time points before/after treatment (P<0.05, **Table 4**).

Correlation between EBC and serum levels of NO in ARDS patients at different time points

Pearson correlation analysis revealed significantly positive correlation between EBC and serum NO levels before treatment, and at 1 day or 5 days after treatment (r = 0.843, 0.821 and 0.862, P = 0.022, 0.026 and 0.017, **Table 5**).

Discussion

ARDS is one acute and progressive respiratory failure caused by intra- and extra-pulmonary factors except cardiac reasons, and is featured with higher vascular permeability of pulmonary circulation, which can induce diffused mesenchymal pulmonary edema and alveolar cavity edema plus progressive hypoxia. Under severe conditions, ARDS can lead to multi-organ dysfunction and compromises patient's life, making the one-month mortality as high as 30% [11]. ARDS has rapid disease progression and high mortality, and is one major dead reason for critical patients [12]. Multiple redox products, soluble markers and inflammatory factors are included in respiratory ductal epithelial lining fluids. With occurrence of basal diseases such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, bronchial dilation, ALI and ARDS, oxidative stress may occur in pulmonary and airway ductal tissues, changing acidic/alkaline status and inflammatory condition, further altering concentrations of multiple biomarkers in respiratory ductal epi-

Table 2. APACHE II score, NO level of EBC and serum in survival and dead patients at different time points

	Survival group			Dead group		
	Before	1 day after	5 days after	Before	1 day after	5 days after
APACHE II score	25.61 ± 7.24	16.73 ± 5.87[a,Δ]	11.53 ± 4.25[a,b,Δ]	26.68 ± 6.95	34.76 ± 6.76[a,Δ]	41.57 ± 5.92[a,b,Δ]
NO content in EBC	48.28 ± 5.61	30.26 ± 2.97[a,Δ]	21.51 ± 3.02[a,b,Δ]	46.52 ± 4.97	49.41 ± 5.62[a,Δ]	55.19 ± 5.59[a,b,Δ]
NO content in serum	47.64 ± 5.94	29.51 ± 3.73[a,Δ]	20.81 ± 3.63[a,b,Δ]	50.35 ± 6.72	52.41 ± 5.63[Δ]	57.56 ± 7.22[a,b,Δ]

Note: [a], comparing between 1 day after and pre-treatment; [b], P<0.05 comparing between 1 day and 5 days after treatment. [Δ], compared to survival group at the same time point.

Table 3. PaO_2/FiO_2, and PaO_2 levels at different time points between survival and dead group

	Survival group			Dead group		
	Before	1 day after	5 days after	Before	1 day after	5 days after
PaO_2/FiO_2 (mmHg)	210.54 ± 19.21	280.29 ± 20.16[a,Δ]	311.42 ± 22.17[a,b,Δ]	209.74 ± 20.11	187.66 ± 19.51[a,Δ]	142.36 ± 18.27[a,b,Δ]
PaO_2 (mmHg)	58.63 ± 5.55	75.57 ± 8.02[a,Δ]	87.97 ± 9.23[a,b,Δ]	59.13 ± 5.83	51.37 ± 6.01[a,Δ]	42.84 ± 5.85[a,b,Δ]

Note: [a], comparing between 1 day after and pre-treatment; [b], P<0.05 comparing between 1 day and 5 days after treatment. [Δ], compared to survival group at the same time point.

Table 4. Correlation analysis between APACHE II score, PaO_2/FiO_2, and PaO_2 levels and NO levels

	NO content in EBC [*r* (*P*)]			Serum NO level [*r* (*P*)]		
	Before	1 day after	5 days after	Before	1 day after	5 days after
APACHE II score	0.483 (0.061)	0.617 (0.041)	0.654 (0.033)	0.592 (0.044)	0.585 (0.052)	0.611 (0.039)
PaO_2/FiO_2	-0.624 (0.029)	-0.595 (0.035)	-0.611 (0.033)	-0.587 (0.038)	-0.682 (0.021)	-0.609 (0.031)
PaO_2	-0.702 (0.020)	-0.658 (0.032)	-0.623 (0.037)	-0.696 (0.039)	-0.711 (0.027)	-0.657 (0.043)

Table 5. Correlation between EBC and serum levels of NO in ARDS patients at different time points

	NO content in EBC					
	Before		1 day after		5 days after	
	r	*P*	*r*	*P*	*r*	*P*
Serum NO level	0.843	0.022	0.821	0.026	0.862	0.01

thelial lining fluids [13, 14]. Therefore, the assay of related biomarker concentration can reflect pulmonary disease conditions. EBC assay is one novel method for examining status of respiratory disease. It is based on the collection of exhalation gas, and can directly analyze biomarkers inside respiratory ductal epithelial lining fluids without septum collection or bronchoalveolar lavage. It thus cannot cause any risk or uncomfortable conditions for patients, making it one novel method to monitor disease condition and treatment efficacy of pulmonary disease due to advantages including non-invasiveness, real-time and repeated tests [15]. As one novel advanced to evaluate airway ductal inflammation and oxidative stress condition, EBC assay has been widely applied due to its advantages including easiness, convenience and repetitiveness [16]. Due to the direct collection of lower respiratory duct, EBC assay has reliability and thus has been widely applied in various developed countries [17].

Over-and dysregulated inflammatory response and oxidative stress-related injury play a critical reason during patho-physiological process [18]. Systemic inflammatory response syndrome (SIRS) is the common pathway and important mechanism of ALI/ARDS pathogenesis. The aggregation of neutrophil, activation and release of proteinase, superoxide and inflammatory, focal release of tumor necrosis factor (TNF) and interleukin (IL) in alveolar macrophage all participate in pathogenesis of ARDS. NO is one recently discovered endogenous small molecule that participates in various body tissue/organs, and can be secreted by endothelium, epithelium and inflammatory cells, and participates in various pathophysiological processes. When ARDS occurs, over-reaction of inflammation inside lung tissues can enhance synthesis of NO synthase (NOS) inside inflammatory, endothelial and airway ductal cells, thus producing abundant NO, to produce $ONOO^{2-}$ with even higher oxidative

toxicity by the rapid reaction with oxygen anion free radicals O^{2-}, inducing lipid peroxidation, causing detachment, dysfunction or even death of epithelial cells, thus aggravating inflammatory response which is closely correlated with ARDS onset and progression [6]. Therefore, inhibition of overexpressed NO synthesis can become one novel method to prevent and correct hypoxia condition in ARDS patients. Moreover, the stress of mechanical ventilation can also aggravate alveolar dilation and inflammation in ARDS patients to different extents, and can further stimulate lung tissues for NO production [19]. Currently, most scholars use serum, IS or BALF samples to evaluate ARDS conditions, whilst EBC has minor usage under such scenarios [8]. This study thus investigated NO content in EBC sample of ARDS patients, in order to provide one non-invasive method to monitor pulmonary oxidative stress and inflammation.

Test results showed significantly higher NO content in EBC and serum samples of ARDS patients compared to healthy control population. Patient NO level was remarkably lowered after treatment but still higher than control group, indicating that EBC or serum NO level can reflect disease condition to certain extents and may work as one reference index for early diagnosis of ARDS. After treatment, body inflammation in ARDS patients was managed, as proved by lower NO synthesis inside lung tissues, suggesting that EBC/serum NO content could reflect the severity of airway ductal inflammation, and might work as the objective index for efficacy evaluation. APACHE II index, EBC/serum NO content began to decrease in survived ARDS patients, with significantly elevated PaO_2/FiO_2, and PaO_2 levels after treatment, indicating relief and correction of hypoxia status, whilst dead group showed unresponsiveness to treatment. Based on the tendency between EBC/serum NO contents and APACHE II score, PaO_2/FiO_2, and PaO_2 levels, certain correlation among EBC/serum NO contents and APACHE II score, PaO_2/FiO_2, and PaO_2 levels in ARDS patients. Further analysis showed significantly positive relationship between EBC/serum NO level and APACHE II score in ARDS patients, plus negative correlation with PaO_2/FiO_2, and PaO_2 levels. Therefore, the assay for EBC/serum NO content effectively reflects hypoxia condition of ARDS patients, and has important implication for clinical treatment and prognostic evaluation. Further analysis revealed positive correlation between EBC NO and serum NO contents in ARDS patients at different time points before and after treatment. Ratnawati et al found that assay of EBC NO level effectively reflected inflammatory status of respirator duct in childhood asthma patients [20]. Ciprandi et al also reported that EBC NO level might help to predict reversibility of respiratory duct in childhood bronchial asthma and allergic rhinitis patients [19]. Liu et al found that EBC NO level can work as the marker for monitoring inflammatory conditions of asthma, COPD, pulmonary cystic fibrosis and lobar pneumonia [21]. This study revealed the role of EBC NO content assay in reflecting hypoxia condition and disease/efficacy evaluation process of ARDS patients, one field that has not been widely studied.

Conclusion

By measuring NO content in EBC, one can directly observe pulmonary hypoxia condition and disease course in ARDS patients. Quantification of EBC NO level can help to evaluate treatment efficacy and determining prognosis. Due to its satisfactory correlation with serum level, EBC NO can work as one index to replace serum NO.

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Disclosure of conflict of interest

None.

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