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ORIGINAL RESEARCH

Relationship Between Endogenous Hydrogen Sulfide and Pulmonary Vascular Indexes on High-Resolution Computed Tomography in Patients with Chronic Obstructive Pulmonary Disease

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Objective: To explore the relationship between endogenous hydrogen sulfide (H_2S) and high-resolution computed tomography (HRCT) indexes in pulmonary vascular remodeling.

Methods: A total of 94 stable chronic obstructive pulmonary disease (COPD) patients were recruited for the study. Plasma H_2S levels were measured using fluorescence probe. Fluorescence quantitative polymerase chain reaction was used to measure H_2S synthase cystathionine-γ-lyase (CSE) mRNA and cystathionine-β-synthesis enzyme (CBS) mRNA. The main pulmonary artery diameter (mPAD), axial diagonal mPAD, coronal mPAD, sagittal mPAD, right pulmonary artery diameter (RPAD), left pulmonary artery diameter (LPAD), and ascending aortic diameter (AAD) and the percentage of total cross-sectional area of vessels less than 5 mm^2 of total lung area (%CSA <5) on HRCT were measured. Pulmonary arterial systolic pressure (PASP) of echocardiography, blood gas analysis, and routine blood tests were performed. Correlation analysis and multivariate linear regression were performed using SPSS 22.0.

Results: H_2S was negatively correlated with mPAD, axial diagonal mPAD, and sagittal mPAD (r = −0.25~−0.32) and positively correlated with PaO_2 (r = 0.35). Relative expression of CSE mRNA was positively correlated with PASP, coronal mPAD, sagittal mPAD, white blood cell count (WBC), and neutrophil count (N) (r = 0.30~0.44). The relative expression of CBS mRNA was positively correlated with PASP, WBC, and N (r = 0.34~0.41). In separate models predicting pulmonary vascular indexes, a 1μmol/L increase in H_2S predicted lower pulmonary artery diameter (for axial diagonal mPAD, 0.76mm lower; for mPAD/AAD, 0.68mm lower). All *P* values were less than 0.05.

Conclusion: Endogenous H_2S may be involved in pulmonary vascular remodeling, providing a new method for the diagnosis and treatment of COPD. The generation of H_2S may be inhibited by hypoxia, inflammation, etc.

Keywords: chronic obstructive pulmonary disease, vascular remodeling, pulmonary hypertension, multidetector computed tomography

Introduction

Pulmonary hypertension (PH) is an important complication of the natural progression of chronic obstructive pulmonary disease (COPD), with a poor prognosis. Although PH is often diagnosed at an advanced stage of COPD, pulmonary vascular remodeling has occurred in patients with early stages of the disease and in smokers without airflow limitation.[1] The main feature of vascular remodeling in

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COPD is thickening of the intimal layers due to vascular smooth muscle (VSM) cell proliferation and deposition of extracellular matrix proteins such as elastin and collagen.[2] Vascular alterations in the pulmonary circulation may impair gas exchange in the alveolar compartments that result in PH in COPD patients.[3] The pathogenesis of pulmonary vascular remodeling in COPD is complex, which can be caused by chronic hypoxia, inflammation, smoking and other factors. Numerous studies have demonstrated the potential role of multiple cytokines in pulmonary vascular remodeling of COPD,[4,5] including vascular endothelial growth factor, endothelin-1, nitric oxide (NO), prostacyclin, transforming growth factor-β, platelet derived growth factor β, matrix metalloproteinase and so no. Some previous studies have explored the efficacy of specific drugs for PH, such as prostacyclin,[6] endothelin receptor antagonists,[7,8] and phosphodiesterase inhibitors[9] in the treatment of COPD-PH, but giving unideal outcome and even results of impairment of oxygenation parameters or quality of life on patients. Oxygen therapy does not prevent the development of PH in COPD patients. Therefore, new biomarkers and therapeutic targets of COPD-PH should be considered.

Hydrogen sulfide (H_2S) is a new type of endogenous gaseous signaling molecule after NO and carbon monoxide. In mammals, H_2S is produced from the substrate L-cysteine, which is catalyzed by H_2S synthase. Three kinds of H_2S synthases, synthase cystathionine-γ-lyase (CSE), cystathionine-β-synthesis enzyme (CBS), and 3-mercaptopyruvate sulfurtransferase are widely distributed in the body and have tissue specificity. Both CSE and CBS are expressed in the lung tissues of mice and are distributed in airway smooth muscle cells, VSM cells, and endothelial cells.[10] H_2S is an important endogenous vasodilator and has been confirmed as a gas opener of the K_{ATP} channel in VSM.[11] Studies have shown that H_2S is a powerful pulmonary artery vasodilator that can significantly reduce PH.[12] Endogenous H_2S was found to be involved in the occurrence of hypoxic,[13] high pulmonary blood flow[14] and monocrotaline-induced[15,16] animal models of PH, and exogenous H_2S could alleviate PH and pulmonary vascular structural remodeling. The mechanism includes relaxation of pulmonary VSM,[12] inhibition of proliferation in VSM cells,[17] induction of apoptosis in VSM cells,[18] inhibition of abnormal accumulation of extracellular matrix in VSM cells,[19] and improvement of antioxidant capacity of lung tissue,[20] etc. H_2S is a potential dilatator of the human pulmonary artery and is an important drug for lowering pulmonary artery pressure.[12] However, the prospect of such treatment needs to be evaluated in further clinical trials.

Finding a convenient and effective way to assess pulmonary vascular remodeling has clinical implications. The invasive nature of right heart catheterization (RHC) and the reduced sensitivity of echocardiography have led to high-resolution computed tomography (HRCT) as an alternative method to diagnose PH. The pulmonary vessel diameter and the cross-sectional area (CSA) of small pulmonary vessels in subsegmental pulmonary vessels of patients with COPD were measured by HRCT to evaluate the remodeling of pulmonary small vessels. Matsuoka's study showed a negative correlation between %CSA < 5 (the percentage of total cross-sectional area of vessels less than 5 mm^2 of total lung area) and % low attenuation area (LAA) as measured by CT.[21] In addition to small pulmonary vessels, the relative enlargement of pulmonary artery on CT was also considered as a marker of pulmonary vascular disease and used to detect the presence of PH. Mahammedi's study showed that a main pulmonary artery diameter (mPAD) > 31.5mm and mPAD/AAD (ascending aortic diameter) >1 may be the best measurements for predicting PH.[22]

This study intended to use HRCT pulmonary vascular indexes as an evaluation index of pulmonary vascular remodeling in patients with COPD, and to study the role of H_2S in it.

Materials and Methods

Subjects

A cross-sectional study approved by the Ethical Committee of the Peking University Third Hospital was conducted. All participants signed an informed consent form. The study was conducted in accordance with the Declaration of Helsinki. A total of 94 stable COPD patients aged 40–85 years who diagnosed in the outpatient department of our hospital from February 2016 to June 2017 were enrolled.

Inclusion criteria met the COPD diagnostic criteria of GOLD 2021: patients with dyspnea, chronic cough or sputum, a history of exposure to risk factors, and forced expiratory volume in one second/forced vital capacity (FEV1/FVC) < 70% after bronchodilator inhalation can be diagnosed as COPD.[23] The stable phase was defined as no significant aggravation of cough, sputum, or dyspnea in the last 2 months. The exclusion criteria were as

follows: (1) other lung diseases such as pneumonia, lung cancer, pulmonary embolism; heart diseases such as heart failure, ventricular septal defect; thoracic deformity; pleural diseases; diabetes, connective tissue disease, hypertension and other diseases that may affect pulmonary vessels. (2) History of pulmonary surgery. (3) Positive bronchodilation test or restrictive ventilation dysfunction.

Questionnaire Survey

The questionnaire survey included information on gender, age, height, weight, smoking status (never smoker, ex-smoker, current smoker), smoking amount, previous history, modified British Medical Research Council Dyspnea Questionnaire (mMRC) score, COPD Assessment Test (CAT) score, and frequency of acute exacerbation of COPD (AECOPD).

Lung Function Measurement

Lung function was determined using a MEDGRAPHICS Profile (USA) spirometer, which included post-bronchodilator the percentage of predicted FEV1 (FEV1%pred) and FEV1/ FVC.

Measurement of Pulmonary Arterial Systolic Pressure (PASP)

PASP of echocardiography was determined using a Vivid 7(GE, USA) color doppler ultrasound scanner. The maximum tricuspid regurgitation velocity was measured by professional physicians in the Department of Cardiac Ultrasound, and the PASP was estimated.[24]

HRCT Measurement of Pulmonary Artery Diameter

A GE Healthcare Advantage Workstation was used to measure the pulmonary artery in the mediastinal window (width 350HU, window position 50HU, layer thickness, 5mm). (1) At the level of the main pulmonary artery (mPA) bifurcation on the axial section, mPAD[25] was measured along the line that originated from the center of the AA and passed perpendicular to the long axis of the main PA. (2) Axial diagonal mPAD[25] was measured on the line that joined the origin of the LPA and the center of the AA on the axial section at the level of the PA bifurcation. (3) Coronary mPAD was measured on the widest short-axis diameter of the mPA on the coronary section. (4) Sagittal mPAD[25] was measured on the widest short-axis diameter of the mPA in the sagittal section. (5) At the widest portion of the right pulmonary artery, right pulmonary artery diameter (RPAD)[25] was measured along the line that originated from the center of the AA and passed perpendicular to the long axis of the right pulmonary artery. (6) At the widest portion of the left pulmonary artery, left pulmonary artery diameter (LPAD)[25] was measured along the line that originated from the center of the AA and passed perpendicular to the long axis of the left pulmonary artery. (7) AAD[25] was measured at the level of the mPA bifurcation. mPAD/AAD was the ratio of mPAD to AAD. Under the guidance of a chief radiologist, the measurement was performed independently by two respiratory physicians using the blind method, and the average value was taken as the final result.

HRCT Measurement of Cross-Sectional Area of Pulmonary Small Vessel

CT measurements of pulmonary small vessels have been described in the literature.[21,26–28] Three slices on the lung window of CT with a thickness of 0.625mm were selected: approximately 1 cm above the upper margin of the aortic arch (the upper cranial slice), approximately 1 cm below the carina (the middle slice), and approximately 1 cm below the right inferior pulmonary vein (the lower caudal slice). Image J 1.48 was used for analysis. "Analyze Particles" function was used to count and measure objects on binary images. After setting specific "size" range of vessels, the number of the vessels and the CSA of each size range on every CT slice were obtained. "Circularity" function was set to analyze the vessels that ran closest to perpendicular to the CT slice based on their shape in the image, excluding vessels that ran obliquely or parallel to the slice. The steps were as follows: (1) The lung field was segmented using a threshold technique with all pixels between −500 and −1024 Hounsfield units (HU) on each CT image. (2) Segmented images were converted into binary images with a window level of −720 HU. Vessels, including pulmonary arteries and veins, were displayed in black on the binary image. (3) We defined vessels with a cross-sectional area of less than 5 mm^2 for the sub-subsegmental area. In the "Analysis Particles" function, we set the "Size" range as 0–5 mm^2 and the "Circularity" range as 0.9–1. The CSA of each vessel was then calculated. We totaled the CSA of vessels measured on each set of three CT slices and obtained a CSA <5, which was the total cross-sectional area of the subsubsegmental vessels that ranged less than 5 mm^2. (4) The total lung area of the three selected slices was obtained using the same

threshold values, with "Size" range set as 0-Infinity and the "Circularity" range set as 0–1.0. Finally, the percentage of CSA < 5 of the total lung area (%CSA < 5) was calculated.

HRCT Measurement of Emphysema

Thoracic VCAR software (GE ADW 4.5) workstation was used to measure the percentage of LAA of each layer lower than −950HU in the CT lung window (layer thickness of 0.625mm), and the average percentage of LAA (% LAA) was obtained.[29]

Measurement of H_2S in Plasma

Blood was collected using a sealed vacuum blood collection tube with heparin sodium anticoagulant and centrifuged at 4000r/min for 5min. The concentration of H_2S was measured using fluorescence probe coated microplates.[30] (1) Ethanol was used to dissolve the fluorescence probe and then added to a 96-well plate. The plate was then placed in a dark room for 1 h. (2) Sodium Hydrosulfide (NaHS) and phosphate-buffered saline were used to prepare H_2S solutions with standard concentrations. Different concentrations of NaHS were added to the probe coated well, and the probe was uncoated well to create a standard curve. (3) A saturated ammonium sulfate buffer was added to the plasma sample and centrifuged to remove the proteins. (4) 100ul supernatant obtained after centrifugation was added to the probe coated well and the probe was uncoated. The plates were placed in an incubator at 37 °C and incubated for 2h. (5) The fluorescence density was acquired at $\lambda_{Ex}/\lambda_{Em}$ 340/445 nm in a microplate reader, the differences in the fluorescence density values between the probe coated well and probe uncoated well were counted, and the H_2S concentration of plasma was calculated using a standard curve with NaHS.

Measurement of CES mRNA and CBS mRNA by Real-Time polymerase chain reaction (PCR)

RNA in blood was extracted using Trizol and reverse transcribed into single-stranded cDNA using MMuLV reverse transcriptase and Oligo (DT)15 Primer. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference. The primers used were as follows: CSE_F: 5′-GATTCGAAAGCCTTGCTGAG-3′, CSE_R: 5′-CCTCATCCTCTAAGCCCACA-3′; CBS_F, 5′- GACCAAGTTCCTGAGCGACA-3′, CBS_R: 5′- CGGAGGATCTCGATGGTGTG-3′; GADPH_F: 5′-TGGGTGTGAACCATGAGAAGT-3′, GADPH_R: 5′-TGAGTCCTTCCACGATACCAA-3′. The amplification multiples of the PCR products were determined according to the amplification curve and melting curve. The difference between the amplification multiples of GAPDH and the PCR products was the relative amplification multiple, which represented the gene expression amount.

Other Tests

A cobas B 123 analyzer (Roche, Switzerland) was used for blood gas analysis. The arterial partial pressure of oxygen (PaO_2) was recorded. Routine blood was detected using a COLTER750 automatic hematology analyzer. The white blood count (WBC) and neutrophil number (N) were recorded.

Data Analysis

Statistical analysis was performed using SPSS 22.0. Enumeration data were expressed as x±s (normal distribution) or M (P25, P75) (non-normal distribution). Categorical data were expressed as the number of cases (percentage). Correlation analysis was performed by Pearson and represented by a scatter diagram. Multivariate linear relationships were analyzed by multivariate linear regression. The "Backward" method was applied to obtain an optimal multiple linear regression equation by removing the insignificant factors one by one from the regression equation containing all selected variables until the last step. Standardized value = (non-standardized value - mean value)/standard deviation. Statistical significance was set at $P < 0.05$.

Results

Basic Clinical Features

The clinical features of patients with COPD were shown in Table 1. There were 94 patients with COPD in the stable phase, including 83 males (88.3%), 11 females (11.7%), 16 never smokers (17.0%), 56 ex-smokers (59.6%), and 22 current smokers (23.4%). Mean age was (69.3±8.9) years, mean mMRC score was 1.6±1.2, mean CAT score was 13.7±7.5, median frequency of AECOPD was 0 (0, 1.0) times, mean FEV1%pred was (51.8±19.6) %, mean FEV1/FVC was (54.1±9.8) %, mean %LAA was (12.5±11.4) %, mean PaO_2 was (73.5±10.7) mmHg, mean count of WBC was (6.5±1.6) $*10^9$/L. Mean plasma H_2S level was (2.6 ±1.6)umol/L. Relative expression of CSE mRNA and CBS

Table 1 Clinical Characteristics of the Subjects

Indexes	Values
Gender (male/ female, n)	83/11
Age (years old)	69.3±8.9
BMI (kg/m^2)	22.6±3.9
Smoking status [n (%)]	
Never smoker	16 (17.0)
Ex-smoker	56 (59.6)
Current smoker	22 (23.4)
Smoking amount (pack years)	33.0 (11.5, 47.0)
mMRC score	1.6±1.2
CAT score	13.7±7.5
Frequency of AECOPD	0 (0, 1.0)
FEV1/FVC (%)	54.1±9.8
FEV1% pred (%)	51.8±19.6
%LAA (%)	12.5±11.4
PaO_2 (mmHg)	73.5±10.7
WBC (*10^9/L)	6.5±1.6
N (*10^9/L)	4.1±1.4
H_2S and Synthetase mRNA	
H_2S (umol/L)	2.6±1.6
Relative expression of CSE mRNA	5.2±4.9
Relative expression of CBS mRNA	3.8±4.4
Intrathoracic vascular indexes on HRCT	
AAD (mm)	36.7±4.0
mPAD (mm)	24.1±4.8
Axial diagonal mPAD (mm)	29.6±4.7
RPAD (mm)	22.1±3.4
LPAD (mm)	22.3±3.6
Coronal mPAD (mm)	29.4±3.3
Sagittal mPAD (mm)	30.0±3.7
mPAD/AAD	0.7±0.1
%CSA<5 (%)	0.30 (0.04, 0.77)
PASP (mmHg)	31.4±11.4

Abbreviations: BMI, body mass index; mMRC, British Medical Research Council Dyspnea Questionnaire; CAT, COPD Assessment Test; AECOPD, acute exacerbation of COPD; FEV1/FVC, forced expiratory volume in one second/forced vital capacity; FEV1%pred, percentage of predicted FEV1; LAA, low attenuation area; PaO_2, arterial partial pressure of oxygen; WBC, white blood count; N, neutrophil number; H_2S, hydrogen sulfide; CSE, synthase cystathionine-γ-lyase; CBS, cystathionine-β-synthesis enzyme; AAD, ascending aortic diameter; mPAD, main pulmonary artery diameter; RPAD: right pulmonary artery diameter; LPAD: left pulmonary artery diameter; CSA, cross-sectional area; PASP, pulmonary arterial systolic pressure.

mRNA were respectively measured in 51 and 52 patients with COPD, and mean values were 5.2±4.9 and 3.8±4.4, respectively. Mean mPAD was (24.1±4.8) mm, and mean mPAD/AAD was 0.7±0.1. Median %CSA<5 was 0.30 (0.04, 0.77)%. PASP was tested in 51 patents with COPD, and mean PASP was (31.4±11.4) mmHg.

Correlation Between H_2S and Basic Clinical Indicators

The correlation of H_2S, CSE mRNA, and CBS mRNA with baseline indicators, such as smoking amount, mMRC, CAT, frequency of AECOPD, PaO_2, WBC, and N were analyzed (Table 2). H_2S was positively correlated with PaO_2 ($r = 0.35$, $P < 0.05$). The relative expression of CSE mRNA was positively correlated with WBC ($r = 0.44$, $P < 0.01$) and N ($r = 0.33$, $P < 0.05$). The relative expression of CBS mRNA was positively correlated with WBC ($r = 0.41$, $P < 0.01$) and with N ($r = 0.34$, $P < 0.05$).

Correlation Between H_2S and Intrathoracic Vascular Indexes

H_2S was negatively correlated with mPAD, axial diagonal mPAD, sagittal mPAD, and AAD ($r = -0.25$, -0.27, -0.32, -0.30, respectively). Relative expression of CSE mRNA was positively correlated with PASP, coronal mPAD, and sagittal mPAD ($r = 0.41$, 0.30, 0.36, respectively). The relative expression of CBS mRNA was positively correlated with PASP ($r = 0.40$). The results are presented in Table 3 and Figure 1.

Multivariate Regression Analysis of H_2S

Using pulmonary vascular quantitative indicators on HRCT as predictors, H_2S, CSE mRNA, and CBS mRNA were analyzed by multivariate linear regression with each vascular index, adjusted by age, sex, body mass index (BMI), smoking amount, PaO_2, and WBC count (Table 4). H_2S entered the optimal multiple linear regression equations for axial diagonal mPAD and sagittal mPAD, respectively. In two different prediction models, the axial diagonal mPAD and sagittal mPAD decreased by 0.763mm and 0.682mm, respectively, for every 1μmol/L increase in H_2S. Relative expression of CSE mRNA was entered into the optimal multiple linear regression equation for coronal and sagittal mPAD respectively. In two different prediction models, coronal and sagittal mPAD were increased by 0.287mm and 0.308mm, respectively, for every 1 increase in relative expression of CSE mRNA. The relative expression of CBS mRNA did not enter the optimal model.

Table 2 Correlation Between H_2S and Basic Clinical Indicators in COPD Patients

Variable	r		
	H_2S (umol/L)	Relative Expression of CSE mRNA	Relative Expression of CBS mRNA
Smoking amount (pack years)	−0.02	−0.21	−0.24
mMRC score	0.05	0.10	0.03
CAT score	−0.09	−0.08	−0.13
Frequency of AECOPD	−0.12	−0.07	−0.09
%LAA (%)	0.03	−0.08	−0.11
PaO_2 (mmHg)	0.35*	0.03	−0.04
WBC (*10^9/L)	0.06	0.44**	0.41**
N (*10^9/L)	0.05	0.33*	0.34*

Notes: *P< 0.05, **P<0.01.
Abbreviations: H_2S, hydrogen sulfide; CSE, synthase cystathionine-γ-lyase; CBS, cystathionine-β-synthesis enzyme; mMRC, British Medical Research Council Dyspnea Questionnaire; CAT, COPD Assessment Test; AECOPD, acute exacerbation of COPD; LAA, low attenuation area; PaO_2, arterial partial pressure of oxygen; WBC, white blood count; N, neutrophil number.

Table 3 Correlation Between H_2S and Intrathoracic Vascular Indexes in COPD Patients

Variable	r		
	H_2S (umol/L)	Relative Expression of CSE mRNA	Relative Expression of CBS mRNA
PASP (mmHg)	−0.10	0.41*	0.40*
mPAD (mm)	−0.25*	0.18	0.15
Axial diagonal mPAD(mm)	−0.27*	0.07	0.06
Coronal mPAD(mm)	−0.11	0.30*	0.20
Sagittal mPAD(mm)	−0.32**	0.36*	0.24
RPAD (mm)	−0.15	0.12	0.11
LPAD (mm)	−0.07	0.03	0.08
AAD (mm)	−0.30**	0.06	0.16
mPAD/AAD (mm)	−0.08	0.08	0.01
%CSA<5 (%)	0.05	−0.07	0.01

Notes: *P< 0.05, **P<0.01.
Abbreviations: H_2S, hydrogen sulfide; CSE, synthase cystathionine-γ-lyase; CBS, cystathionine-β-synthesis enzyme; PASP, pulmonary arterial systolic pressure; mPAD, main pulmonary artery diameter; RPAD: right pulmonary artery diameter; LPAD: left pulmonary artery diameter; AAD, ascending aortic diameter; CSA, cross-sectional area.

Discussion

As a novel gaseous signaling molecule with cardiovascular regulatory functions, H_2S has a variety of cardiovascular effects, including relaxation of VSM, lowering blood pressure and inhibiting smooth muscle cell proliferation. The down-regulation of H_2S pathway is involved in the pathogenesis of a variety of vascular diseases, such as hypertension, atherosclerosis and pulmonary hypertension.[31] It suggests that H_2S is involved in the regulation of the proliferation of pulmonary artery smooth muscle cells. H_2S may play a protective role in pulmonary vascular structural remodeling and PH in rats with high pulmonary blood flow by inhibiting the NO/ nitric oxide synthase pathway and enhancing the carbon monoxide/heme oxygenase pathway.[32] After administration of exogenous H_2S donor NaHS in rats with high pulmonary blood flow, the proliferation of pulmonary artery smooth muscle cells and the ratio of phosphorylated extracellular signal-regulated kinase (PERK)/ERK1 protein expression in pulmonary arteries decreased significantly, suggesting that exogenous H_2S may inhibit the proliferation of pulmonary artery smooth muscle cells induced by high pulmonary blood flow stimulation via the mitogen-activated protein kinase /ERK signal transduction pathway.[33] Research has shown that the degree of small resistance pulmonary artery medial wall thickness, full vascular muscularization, proliferation marker proliferating cell nuclear antigen, and oxidative stress marker 3-neurotrophin in cigarette smoke (CS) group were significantly higher than those in the control group and the CS + NaHS group in rats. It has been

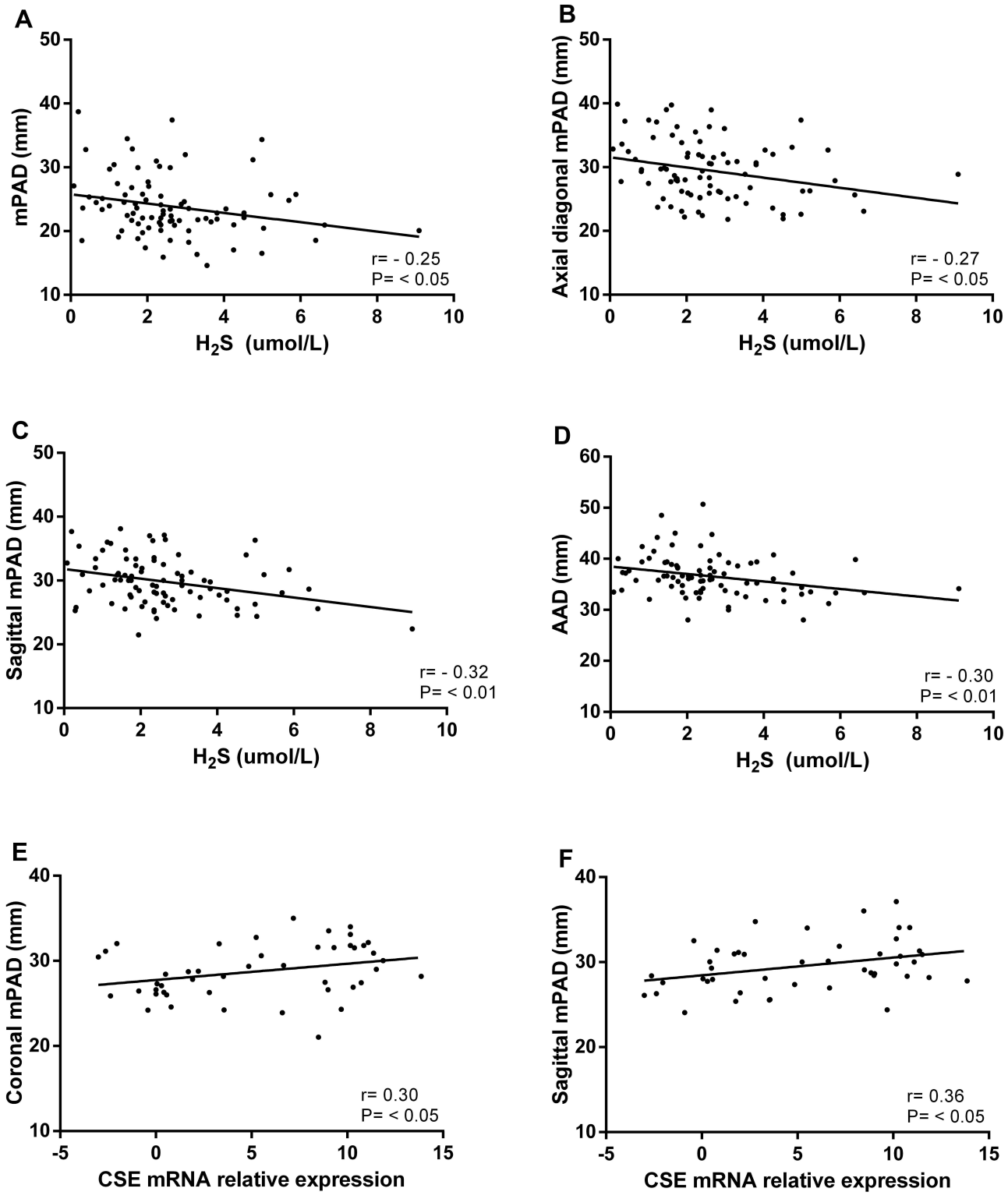

Figure 1 Correlation and scatter plot of hydrogen sulfide (H_2S), synthase cystathionine-γ-lyase (CSE) mRNA and pulmonary vascular indexes on HRCT. (**A**) H_2S was negatively correlated with main pulmonary artery diameter (mPAD) (r = - 0.25, P < 0.05); (**B**) H_2S was negatively correlated with axial diagonal mPAD (r = - 0.27, P < 0.05); (**C**) H_2S was negatively correlated with sagittal mPAD (r = - 0.32, P < 0.01); (**D**) H_2S was negatively correlated with ascending aortic diameter (AAD) (r = - 0.30, P < 0.01); (**E**) Relative expression of CSE mRNA was positively correlated with coronal mPAD (r = 0.30, P < 0.05); (**F**) Relative expression of CSE mRNA was positively correlated with sagittal mPAD (r = 0.36, P < 0.05).

Table 4 Multivariate Linear Regression Analysis of H_2S in Patients with COPD

Dependent Variable	Independent Variable	Unstandardized Coefficients B	Standard Error	Standardized Coefficients β	t	P
Axial diagonal mPAD	H_2S	−0.763	0.375	−0.279	−2.037	0.048
Sagittal mPAD	H_2S	−0.682	0.288	−0.325	−2.368	0.022
Coronal mPAD	Relative expression of CSE mRNA	0.287	0.111	0.416	2.576	0.015
Sagittal mPAD	Relative expression of CSE mRNA	0.308	0.100	0.468	3.086	0.004

Abbreviations: H_2S, hydrogen sulfide; CSE, synthase cystathionine-γ-lyase; CBS, cystathionine-β-synthesis enzyme; mPAD, main pulmonary artery diameter.

suggested that exogenous H_2S can significantly attenuate pulmonary vascular remodeling in smoke-induced COPD, which may be related to the reduction of oxidative stress injury.[20] Han et al[34] also showed that H_2S could inhibit oxidative stress, airway inflammation, and pulmonary vascular remodeling caused by smoking, and ameliorate the development of emphysema and PH. Under hypoxia, H_2S reduced the synthesis of collagen and elastin, thereby inhibiting excessive accumulation of extracellular matrix of pulmonary artery and alleviating hypoxic pulmonary vascular structural remodeling.[35]

Pulmonary vascular remodeling is an important pathological process in the development of COPD to PH and cor pulmonale. Chest CT could be a simple and effective modality for the diagnostic evaluation of COPD and PH.[36] In this study, pulmonary vascular indexes on HRCT were used as the evaluation marker of pulmonary vascular remodeling to study the role of H_2S. The results of this study showed that plasma H_2S was negatively correlated with mPAD, axial diagonal mPAD, and sagittal mPAD. This suggested that endogenous H_2S may be involved in pulmonary vascular remodeling in patients with COPD. Ariyaratnam et al showed that 500 μm H_2S caused a dilation of 42.3% in isolated arterial rings and a 17.7% reduction in pulmonary artery pressure.[12] Zhang et al found that H_2S significantly reduced pulmonary arterial pressure and alleviated pulmonary vascular structural remodeling in rats with hypoxic pulmonary hypertension.[37] It has been suggested that H_2S has a protective effect on pulmonary vascular remodeling.

The relative expression of CSE mRNA in blood was positively correlated with PASP, coronal mPAD, and sagittal mPAD, and CBS mRNA was positively correlated with PASP in COPD patients. These results indicated that with the aggravation of pulmonary vascular remodeling, the relative expression of CSE mRNA and CBS mRNA increased, which may be a compensatory protective mechanism in the pathological state to repair the impaired expression of H_2S. This is consistent with our previous study showing that CSE protein decreased, but CSE mRNA significantly increased in the lung tissues of COPD patients.[38] The correlation coefficients between H_2S, CSE mRNA, CBS mRNA and pulmonary vascular indexes on HRCT were not high. It might be due to that the indexes on HRCT were surrogate markers of mean pulmonary arterial pressure (mPAP) on RHC which was the gold standard of PH.

%CSA < 5 was not related to plasma H_2S, the relative expression of CSE mRNA and CBS mRNA in our study. We speculated that the possible reason was that we chose only three CT slides to calculate the cross-sectional area of small pulmonary vessels, which could not fully represent all small pulmonary vessels in lung to reflect vascular remodeling or pruning. Estépar et al calculated total blood vessel volume (TBV) and the aggregate blood vessel volume for vessels less than $5mm^2$ (BV5) of lung, and found that smoking-related COPD was characterized by distal pruning of the small blood vessels and loss of tissue in excess of the vasculature.[39] George R Washko's study revealed that pulmonary arterial pruning measured by arterial BV5 was associated with clinically significant increases in RV volume in smokers with COPD and is related to exercise capacity and mortality in COPD.[40] BV5 or BV5/TBV might be a more comprehensive and reasonable measure to assess the small blood vessels in lung. Further studies can explore the relationship between H_2S and BV5 or BV5/TBV.

In this study, H_2S was positively correlated with PaO_2, suggesting that hypoxia may have an inhibitory effect on H_2S. Zhang et al revealed that mPAP increased, the plasma

level of H_2S decreased, and the activity of CSE and relative CSE mRNA decreased in the lung tissue of rats in the hypoxia group compared with that in the control group, suggesting that hypoxia had an inhibitory effect on the endogenous H_2S system in rats.[41] Another study showed that H_2S in the lungs of rats in the hypoxic group was significantly decreased compared with that in the control group. After treatment with sodium hydrosulfide, compared with the hypoxic group, pulmonary arterial pressure was decreased by 31.2%, oxidized glutathione was decreased by 23%, and total antioxidant capacity was increased by 19%, suggesting that H_2S plays an important role in the regulation of oxidative stress in hypoxic PH.[13]

Neutrophilic inflammation is an important factor in the development of airway obstruction. The relative expression of CSE mRNA and CBS mRNA in plasma was positively correlated with WBC and N, respectively, suggesting that elevated CSE mRNA and CBS mRNA play a compensatory role in severe inflammation. Our previous research showed that H_2S levels in the plasma of COPD patients were negatively correlated with the proportion of sputum eosinophils, and positively correlated with the proportion of lymphocytes and macrophages. Compared with stable COPD patients, H_2S in plasma was lower in AECOPD, but the proportion of sputum neutrophils was increased, suggesting that H_2S has a potential role in regulating the inflammatory response in different stages of COPD.[42] H_2S attenuated pulmonary artery endothelial cell inflammation in rats with monocrotaline-induced PH by inhibiting the nuclear factor kappa-B pathway.[15] Exogenous H_2S could be a potential drug to improve diabetic impaired wound healing by attenuating inflammation (neutrophils, macrophages, tumor necrosis factor-α, interleukin-6) and increasing angiogenesis.[43]

The predictive effects of H_2S, CSE mRNA, or CBS mRNA on pulmonary vascular indicators were analyzed after adjusting for confounding factors such as age, sex, BMI, smoking amount, PaO_2, and WBC, with pulmonary vascular quantitative indicators on HRCT as effect indicators. In multiple optimal prediction models, increased H_2S predicted lower axial diagonal mPAD and sagittal mPAD, and increased relative expression of CSE mRNA predicted higher coronal and sagittal mPAD. This indicated that the change of H_2S in COPD patients could predict the change in pulmonary vascular indexes, which further suggested that H_2S might play a role in pulmonary vascular remodeling. H_2S has a variety of physiological effects on the blood vessel walls. As this field continues to grow, it is expected that H_2S-related compounds will enter clinical trials to treat diseases that affect blood vessels.[44]

Conclusion

In summary, our study found that H_2S was negatively correlated with pulmonary artery diameter on HRCT in COPD patients. Relative expression of CSE mRNA was positively correlated with pulmonary artery diameter on HRCT and PASP, and relative expression of CBS mRNA was positively correlated with PASP. H_2S may play an important role in pulmonary vascular remodeling in COPD patients, providing a basis for the development of new therapeutic targets. H_2S was positively correlated with PaO_2, while the relative expression of CSE mRNA and CBS mRNA was positively correlated with WBC and N respectively, suggesting that hypoxia and inflammation might inhibit the generation of H_2S. The molecular signaling pathway of H_2S in the occurrence and development of PH in COPD has not been fully elucidated, and no clinical trials related to H_2S to treat PH have been carried out to date. Further exploration of the signaling pathway of H_2S in pulmonary vascular remodeling in COPD and the development of clinical trials related to H_2S treatment is required as it will shed new light on the treatment of COPD-PH, to reduce the morbidity and mortality of COPD - PH.

Abbreviations

PH, pulmonary hypertension COPD, chronic obstructive pulmonary disease; VSM, vascularsmooth muscle;NO, nitric oxide; H_2S, hydrogen sulfide; CSE, synthase cystathionine-γ-lyase; CBS, cystathionine-β-synthesis enzyme; RHC, right heart catheterization; HRCT, high-resolution computed tomography; CSA, cross-sectional area of small pulmonary vessels; LAA, low attenuation area; mPAD, main pulmonary artery diameter; AAD, ascending aortic diameter; FEV1/FVC, forced expiratory volume in one second/forced vital capacity; mMRC, British Medical Research Council Dyspnea Questionnaire; CAT, COPD Assessment Test; AECOPD, acute exacerbation of COPD; FEV1%pred, percentage of predicted FEV1; PASP, pulmonary arterial systolic pressure; mPA, main pulmonary artery; RPAD, right pulmonary artery diameter; LPAD, left pulmonary artery diameter; NaHS, sodium hydrosulfide; PCR, polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PaO_2, arterial partial pressure of oxygen; WBC, white blood count; N, neutrophil number; BMI, body mass index; PERK, phosphorylated extracellular signal-regulated kinase; CS, cigarette smoke; mPAP, mean pulmonary

arterial pressure; TBV, total blood vessel volume; BV5, aggregate blood vessel volume for vessels less than 5mm^2.

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Disclosure

The authors report no conflicts of interest in this work.

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