


Serum H₂S as an indicator of exacerbation and pulmonary arterial hypertension in chronic obstructive pulmonary disease

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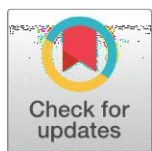
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ABSTRACT

Background and objective: Hydrogen sulfide (H₂S) is a potential gasotransmitter produced by respiratory and vascular smooth muscle cells. Although some studies have examined H₂S in chronic obstructive pulmonary disease (COPD), few have investigated its role in COPD exacerbations and pulmonary arterial hypertension (PAH). This study aimed to compare serum H₂S levels between COPD patients and healthy controls, examine associations between H₂S and COPD severity/exacerbations/PAH, and compare H₂S levels between smokers and nonsmokers.

Methods: Serum H₂S was measured in 16 patients with stable COPD and 34 with acute exacerbations. COPD severity was classified using GOLD stages. Arterial blood gases, pulmonary arterial pressure by echocardiography, and clinical variables were assessed. Multivariable regression analyzed factors influencing H₂S. ROC curves evaluated the diagnostic utility of H₂S for exacerbations and PAH.

Results: Serum H₂S was lowest in GOLD stage IV patients compared to stages II and III. Levels were significantly lower in acute exacerbations versus stable COPD. COPD patients with PAH had lower H₂S than those without. Arterial pH, FEV₁, and FEV₁/FVC positively associated with H₂S, while Pa_{CO2}, severity, exacerbations, and PAH negatively influenced H₂S. Optimal H₂S cutoffs for indicating exacerbations and PAH were <46.7 μmol/L.

Conclusions: Serum H₂S may be a useful indicator of exacerbations and PAH in COPD patients.

Keywords COPD, Gasotransmitter, Hydrogen sulfide, PAH, sPAP

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disease of the lungs characterized by dynamic hyperinflation, parenchymal damage and irreversible airflow limitation.¹ According to the World Health Organization global burden of disease studies, COPD ranks third in terms of mortality and morbidity globally, and is expected to step up to the second leading cause of mortality by 2030.² Episodes of exacerbation are one of the major physiological manifestations of COPD which is often associated with disease severity, cardiac comorbidity, impaired quality of life and mortality.³ Pulmonary arterial hypertension (PAH) is a comorbidity in COPD that is observed among patients in advanced stages of COPD, presumably when the gas exchange is severely compromised. In addition to that, hypoxic vasoconstriction, collagen deposition, remodeling of the pulmonary arteries and emphysematous changes in the capillary bed eventually lead to pulmonary hypertension and right ventricular dysfunction.^{4,5}

In recent years, a number of molecules have been investigated to play crucial role in COPD pathophysiology. Hydrogen sulfide (H₂S) is one of those molecules. H₂S is a gas with a typical rotten egg smell, and it is a potential gasotransmitter synthesized in the pulmonary arterial and airway smooth muscle cells, endothelial cells and primary fibroblast by cystathionine- γ -lyase (CSE), cystathionine- β -synthase (CBS) and 3-mercaptopyruvate sulfur transferase in mammals.⁶ H₂S has been shown to deliver potential anti-inflammatory and antioxidant effects by researchers in animal model.⁷⁻⁹ In clinical studies, some researchers demonstrated higher levels of serum H₂S in COPD in compared to healthy non-smokers although H₂S level was reduced in patients with acute exacerbation.^{3,10} The levels of serum H₂S have also been tested in certain cardiovascular conditions associated with COPD.¹¹ Although there is only one study that demonstrated a lower serum H₂S among the COPD patients with pulmonary arterial systolic pressure ≥ 35 mmHg.¹⁰ No other study describes serum H₂S levels in pulmonary arterial hypertension.

In this study, we aimed at investigating the serum H₂S level in patients with COPD and COPD patients with PAH. We also evaluated whether serum H₂S level could indicate the occurrence of exacerbation and PAH in COPD.

MATERIALS AND METHODS

Study participants

Fifty COPD patients attending the indoor and outdoor specialty clinics of the Department of Respiratory Medicine of the NRS Medical College and Hospital in Kolkata were recruited for the study. Among the 50 patients, 23 had GOLD Stage 4 disease, 13 patients had stage 3, and 14 patients had Stage 2 disease. Among the cases, 44 were smokers and 4 were exposed to ETS. Parameters were matched with age- and sex-matched apparently healthy controls selected from relatives of the patients and hospital employees. The study

was conducted over a period of one year from April 2014 to March 2015. Among the controls, 13 were smokers and 37 were non-smokers.

Inclusion and exclusion criteria

Patients were excluded if they a) had clinical, laboratory or radiological evidence of pneumonia, pulmonary tuberculosis, bronchiectasis, bronchogenic carcinoma, asthma, interstitial lung disease or pregnancy; b) were on H₂S donor or H₂S inhibitor drugs; or c) did not provide informed consent.

After all exclusions, 50 patients were recruited for the study. The diagnosis of COPD was made according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.¹²

Patients with a history of chronic respiratory symptoms such as dyspnea, coughing, sputum production or both, a smoking history of >10 pack-years, and a post-bronchodilator FEV₁/FVC ratio <70% were recruited as cases. Disease severity of the patients was assessed by GOLD stages I to IV. Patients were categorized as stable COPD (S-COPD) if they did not have any symptoms of exacerbation in the last 4 weeks, or with acute exacerbation (AECOPD) if they reported new or worsening respiratory symptoms (cough, dyspnea and/or sputum production) for two or more consecutive days.¹³

Sample size

The average patient volume of COPD in outpatient departments and those admitted with AECOPD was calculated for the past 3 years, and since the prevalence of COPD is 9.23% in this region of India, the sample size was calculated with 10% allowable error. Due to funding and time constraints, a larger sample size could not be obtained. We planned to work with a bigger sample in the future over a longer timeframe.

Echocardiography

Doppler echocardiography (DE) was performed to measure the pulmonary arterial systolic pressure (sPAP) in a non-invasive way by using conventional echocardiographic equipment (GE Healthcare, Model: Vivid T8 and P3, USA) with 3Hz transducer mode. Pericardium and cardiac functions including the valvular anatomy and function and chamber size, were assessed from the echocardiograms. Tricuspid regurgitant (TR) flow was identified by color flow Doppler procedure and the TR jet was obtained in Parasternal long-axis/RV inflow, parasternal short axis at the level of aortic valve, apical four-chamber, and subcostal views. The measurement of modal peak velocity was obtained by maximum TR jet velocity where the Doppler envelop was complete. Right ventricular systolic pressure (sRVP) was considered to be equal to the sPAP in the absence of an obstruction in the

right ventricular outflow tract; and the sPAP was estimated based on the modified Bernoulli equation: $sPAP_{mmHg} = \text{right ventricular systolic pressure} = \text{trans-tricuspid gradient} + \text{right atrial pressure (RAP)}$ where trans-tricuspid gradient is $4v^2$ ($v = \text{peak velocity of tricuspid regurgitation, m/second}$).¹⁴⁻¹⁷

Arterial blood gas analysis

Arterial blood gas samples were analyzed in an ABL90 Flex blood analyzer (Radiometer, Bengaluru, India) according to established guidelines.¹⁸ The pH, partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were recorded.

Measurement of serum H₂S

Serum H₂S was measured according to a protocol reported elsewhere with minor modifications and standardization.^{19,20} This spectrophotometric method is based on the formation of methylene blue from the reactions of a sulfide salt with N,N-dimethyl-p-phenylenediamine sulfate in the presence of oxidizing Fe^{3+} in an acidic medium. The absorbance of methylene blue was measured at 670nm wavelength using a UV-Vis dual beam spectrophotometer (Systronics India Ltd, New Delhi, India).

Method of measurement of H₂S concentration in serum:

This spectrophotometric method involves the reaction of sulfide with N,N-dimethyl-p-phenylenediamine sulfate in the presence of the oxidizing agent Fe^{3+} in hydrochloric acid to form methylene blue, which is read at 670nm.

Assay procedure: Seventy-five microliters of serum was added to 425 microliters of phosphate buffered saline (PBS) and 250 microliters of 10% trichloroacetic acid in a capped glass tube. It was then centrifuged at 3000 rpm for 30 minutes. The supernatant was taken in another glass tube, and 250 microliters of 1% zinc acetate, 133 microliters of 20 millimolar N,N-dimethyl-p-phenylenediamine sulfate in 7.2 mM HCl, 133 microliters of 30 millimolar $FeCl_3$ in 1.2 mM HCl, and 60 microliters of 10% NaOH were added, capped, and incubated for 10 minutes at room temperature. All samples were assayed in triplicate, and serum H₂S levels were calculated against a calibration curve prepared with 25-250 micromol/L concentrations of sodium sulfide (NaHS, Sigma-Aldrich, MO, USA) (previously published by some of the authors in a different H₂S study). The intra-assay and inter-assay variations of this method were 7.576 and 3.944 respectively, and the maximum sensitivity was up to 25 micromol/L.²¹

Statistical analysis

Data were depicted as mean and SD for normally distributed variables and median with interquartile range (IQR) for non-normally distributed variables. To compare serum H₂S levels between S-COPD and AECOPD and between COPD patients with and without PAH,

student's t-tests were performed. One-way analysis of variance (ANOVA) was used to test serum H₂S levels across GOLD stages, and Bonferroni's test was used to assess intergroup variation. To evaluate the association between serum H₂S level and parameters related to COPD and PAH, we first tested the bivariate relationship between serum H₂S and each clinical parameter using student's t-test and Spearman's rank order correlation as appropriate. Second, to assess associations between possible confounders such as age, sex, smoking status, BMI, and serum H₂S and all clinical parameters, we performed the aforementioned tests (Student's t-test, Spearman's rank order correlation, and Wilcoxon signed rank test if required). Third, a multivariable linear regression model was constructed for serum H₂S against each clinical variable if those variables exhibited a *p*-value <0.2 in the corresponding bivariate analysis. Covariates were included in the model using a step-forward and step-backward selection process if (i) they related to both the exposure and outcome in bivariate analysis, (ii) caused >10% change in the regression coefficient estimates of the remaining variables in the multivariable models, or (iii) were found to associate with serum H₂S level in previously published literature. Regression diagnostics were performed to assess model goodness-of-fit and eliminate any possible collinearity between clinical variables.

Receiver operating characteristic (ROC) curves were constructed to determine the predictive value of serum H₂S for acute exacerbations and occurrence of PAH in COPD. For all analyses, a two-tailed *p*-value <0.05 was considered significant. All analyses were performed in STATA V.12.0 (Stata Corp, College Station, Texas, USA).

RESULTS

Characteristics of the patients

The baseline demographic characteristics of the patients are presented in Table 1. There were 45 male patients and 5 female patients, all with a mean body mass index of 21.1±3.7 kg/m². According to the GOLD staging for severity, 28%, 26% and 46% of the patients belonged to GOLD-II, III and IV, respectively (no patients were in GOLD-I). Thirty-four patients (68%) had AECOPD and 16 (32%) had stable COPD (S-COPD). ABG analysis demonstrated a mean arterial blood pH of 7.36±0.09, *P*aO₂ of 76.5±17.8 mmHg and *P*aCO₂ of 67.5±20.2 mmHg. The patients had a mean pulmonary arterial systolic pressure (sPAP) of 36.3±16.4 mmHg obtained from the DE test and 37 (74%) patients were diagnosed with PAH. Mean serum H₂S of the patients was 40.4±15.1 μmol/L.

Table 1. Demographic and clinical and biochemical profiles of the study participants.

	All COPD patients (N=50)
Age (years), mean (SD)	61.3 (9.6)
Gender, male (%)	45 (90)
Weight (Kg), mean (SD)	53.6 (10.1)

Continued on next page

Table 1 continued

	All COPD patients (N=50)
Height (meter) , mean (SD)	1.6 (0.04)
BMI (kg/m²) , mean (SD)	21.1 (3.7)
FEV1 (% predicted) , mean (SD)	43.9 (18.1)
FEV1/FVC , mean (SD)	0.55 (0.09)
GOLD stage , n (%)	
Stage 1	0 (0)
Stage 2	14 (28)
Stage 3	13 (26)
Stage 4	23 (46)
Exacerbation , n (%)	
S-COPD	16 (32)
AECOPD	34 (68)
Arterial blood pH , mean (SD)	7.36 (0.09)
PaO₂ (mmHg) , mean (SD)	76.5 (17.8)
PaCO₂ (mmHg) , mean (SD)	67.5 (20.2)
sPAP (mmHg) , mean (SD)	36.3 (16.4)
Pulmonary arterial hypertension , n (%)	37 (74)
Serum H₂S (μmol/L) , mean (SD)	40.4 (15.1)

Data presented in the table as mean (SD), median (25th-75th percentile) or frequency (%), unless otherwise mentioned. Abbreviations: BMI: body mass index; PASP: pulmonary artery systolic pressure; FEV₁: forced expiratory volume in 1 second; GOLD: global initiative for chronic obstructive pulmonary disease; S-COPD: stable COPD; AECOPD: acute exacerbated COPD; PaO₂: partial pressure of oxygen; HCO₃⁻: bicarbonate ion; H₂S: hydrogen sulphide.

Serum H₂S level

Serum H₂S level differed significantly (overall ANOVA $p < 0.001$) across the GOLD stages of COPD severity. Patients in GOLD-IV stage had the lowest level of serum H₂S ($31.8 \pm 11.8 \mu\text{mol/L}$) compared to GOLD-II and GOLD-III (51.1 ± 12.6 vs. $44.1 \pm 14.5 \mu\text{mol/L}$, respectively; both $p < 0.01$) (Figure 1). We also observed that H₂S level was significantly ($p = 0.008$) lower among the AECOPD patients ($36.6 \pm 2.5 \mu\text{mol/L}$) compared to the S-COPD patients ($48.4 \pm 3.5 \mu\text{mol/L}$) (Figure 2). After stratifying the patients based on the co-occurrence of PAH, we observe

d that the COPD patients who were diagnosed with PAH had a significantly lower serum H₂S level than those without PAH (34.6 ± 2.0 vs. $56.9 \pm 2.7 \mu\text{mol/L}$, $p < 0.001$) (Figure 2).

Relationship between serum H₂S and clinical parameters

In Table 2, we present the relationships between serum H₂S and the clinical parameters relevant to COPD. Arterial blood pH was found to have a strong correlation with serum H₂S level ($p < 0.001$). Both FEV₁ (% predicted) and FEV₁/FVC ratio demonstrated positive associations with serum H₂S and the estimates did not change after adjusting for potential

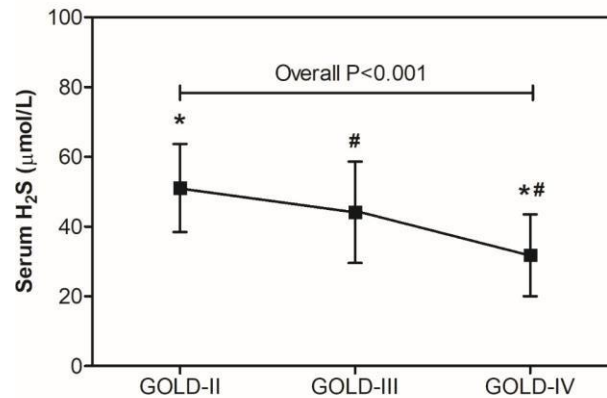


Figure 1 Concentration of serum H₂S across COPD stages, GOLD-II, III and IV. * indicates difference between GOLD-II and IV and # indicates difference between GOLD-III and IV. Level of significance $p < 0.05$. (For abbreviation, see abbreviations list.)

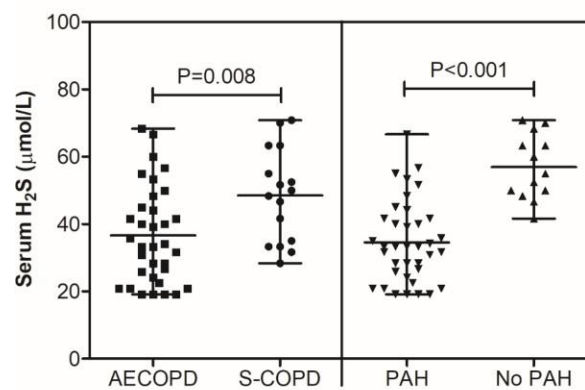


Figure 2 Level of serum H₂S (i) between S-COPD and AE-COPD, and (ii) with and without PAH. (For abbreviation, refer to the list).

confounders such as age, gender, smoking history and BMI. $PaCO_2$ demonstrated a negative association (regression coefficient β : -0.46; 95% confidence interval: -0.61 to -0.31) with serum H₂S in a multivariable model after adjusting for potential confounders. Serum H₂S level was found to be negatively associated with the GOLD severity stages (-9.4; -13.3 to -5.4) and acute exacerbation in COPD (-10.5; -18.8 to -2.3). We also observed that the co-occurrence of PAH also negatively influenced serum H₂S level significantly (-19.2; -26.3 to -12.1).

Table 2. Multiple linear models for the clinical parameters associated with serum H₂S.

	Unadjusted model		Adjusted model	
	β (95% CI)	<i>P</i>	β (95% CI)	
Blood pH	138.2 (110.0 to 166.3)	<0.001	123.1 (93.8 to 152.4)	<0.001
<i>P</i> aCO ₂ (unit)	-0.52 (-0.68 to -0.37)	<0.001	-0.46 (-0.61 to -0.31)	<0.001
FEV1 (% predicted)	0.47 (0.27 to 0.67)	<0.001	0.41 (0.22 to 0.60)	<0.001
FEV1/FVC	1.1 (0.7 to 1.4)	<0.001	1.2 (0.8 to 1.5)	<0.001
GOLD stage	-9.8 (-14.1 to -5.5)	<0.001	-9.4 (-13.3 to -5.4)	<0.001
Exacerbation	-11.8 (-20.5 to -3.2)	<0.001	-10.5 (-18.8 to -2.3)	0.014
sPAP (mmHg)	-0.69 (-0.87 to -0.52)	<0.001	-0.63 (-0.83 to -0.43)	<0.001
PAH	-22.4 (-29.8 to -14.9)	<0.001	-19.2 (-26.3 to -12.1)	<0.001

Data presented as regression coefficient and 95% confidence interval (CI) unless otherwise specified. Each row indicates a single model. Each model is adjusted for age, gender, smoking history and BMI.

Serum H₂S and sPAP

We found that serum H₂S level was negatively influenced by sPAP (R^2 : 0.57; p <0.001, Figure 3). In an unadjusted model, we observed a 0.69 $\mu\text{mol/L}$ decline of serum H₂S with a per unit increase of sPAP, the estimate remained unchanged upon adjustment for potential confounders (-0.63; -0.83 to -0.43).

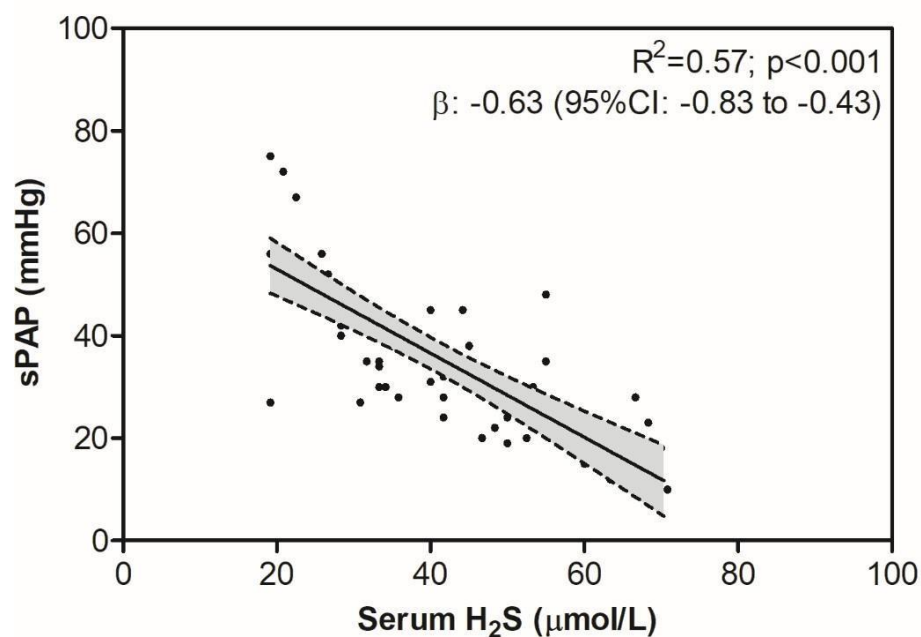


Figure 3 Relationship between serum H₂S level and sPAP. R^2 = coefficient of determination, β = regression coefficient, 95% CI= 95% confidence interval. (For abbreviation, see the list).

H₂S ratio as indicative of an exacerbation and PAH

We constructed a ROC curve to determine the cut-off level of serum H₂S as an indicator of acute exacerbation from stable COPD (Figure 4). Since higher serum H₂S level was associated with more stable COPD (conversely, reduced exacerbation), the ROC was constructed using negative H₂S as the indicator variable. The optimal cut-off level of H₂S for indicating an exacerbation was <46.7 $\mu\text{mol/L}$ (area under the curve: 0.730, 95% CI: 0.59-0.87). A similar ROC was constructed to determine PAH in COPD (Figure 5). We found an optimal cut-off value for H₂S indicating the possible occurrence of PAH <46.7 $\mu\text{mol/L}$ (AUC: 0.917; 95%CI: 0.84-0.99).

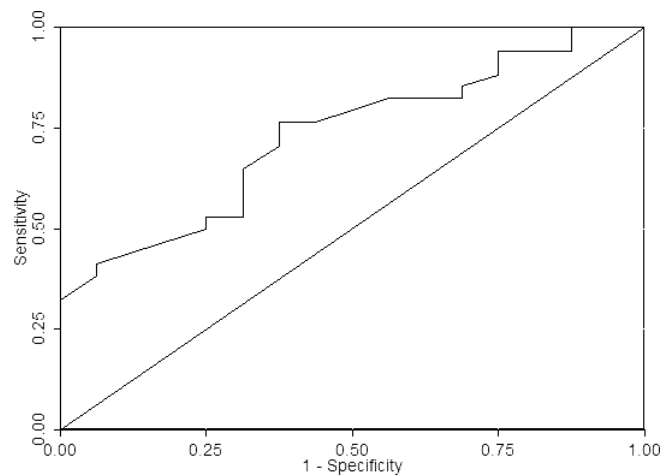


Figure 4 Receiveroperator characteristics (ROC) curve for H₂S to predict the risk ofexacerbation in COPD.

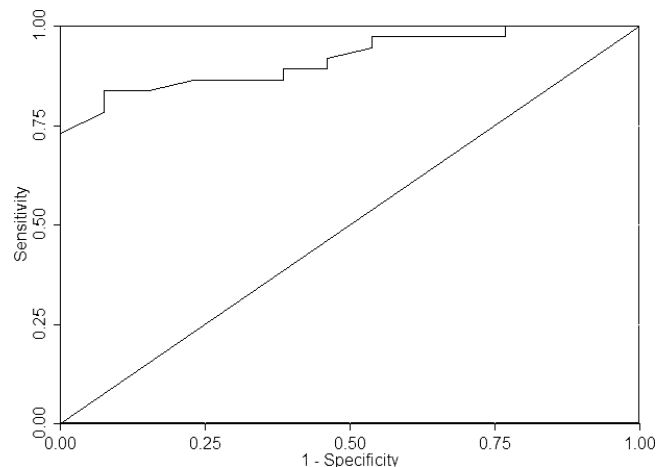


Figure 5 Receiver operator characteristics (ROC) curve for H₂S to predict the risk ofdeveloping PAH among patients with COPD.

DISCUSSION

In this study, we observed that serum H₂S level was lower among the COPD patients with acute exacerbation in compared to the stable-COPD patients. There was a consistent drop of serum H₂S level in accordance with the increase of disease severity, i.e., serum H₂S level was the lowest among the patients in GOLD-IV stage in compared to those in GOLD-II and GOLD-III. Among the COPD patients with co-occurrence of PAH, the serum H₂S level was significantly lower than the H₂S level in those COPD patients without PAH. Serum H₂S level had a significant inverse correlation with PaCO₂ and exacerbation and positive correlations with lung functions suggesting that serum H₂S level is an important determinant of exacerbation and associated clinical manifestations in COPD. The strong inverse relation between sPAP and serum H₂S also indicated that serum H₂S could be a useful indicator of PAH.

The role of endogenously synthesized H₂S in COPD has been reported in only a few research articles. We observed that FEV1% and FEV1/FVC were positively correlated with serum H₂S which is consistent with previously published reports.^{10,11} Our result of decrease in level of H₂S in according to the disease severity also substantiates previous report.¹⁰ Our observation of markedly reduced serum H₂S level among the AECOPD patients than the S-COPD patients are also in accordance with the previously published reports.^{3,10} It has been demonstrated that the serum H₂S drops in conditions, like chest infection.²² Also, in animal models, deficiency of H₂S synthesizing enzymes was found to associate with increased airway hyper-responsiveness inflammation.^{7,23} These observations speak on the role of endogenous H₂S in COPD pathobiology.

H₂S has been demonstrated to have potentially important role in vasodilation of the pulmonary arteries and reducing pulmonary arterial pressure^{24,25} although much is unknown about the true mechanism. In COPD, pulmonary fibrosis and obstructive sleep apnea, the development of global pulmonary hypoxia is a clinically important perturbation that may lead to hypoxic pulmonary hypertension.²⁶ However, there is only one study reported so far on the possible role of H₂S in COPD-associated PAH. Both in our results and that of the previously published one, the COPD patients diagnosed with PAH had a lower serum H₂S level than those COPD patients without PAH.¹⁰ In isolated human lungs model, exogenous administration of H₂S substantially reduced pulmonary artery pressure.²⁵ In animal model, the effect of H₂S on pulmonary arteries has been well demonstrated. In an artificially developed PAH model in rats, H₂S donor was observed to reduce pulmonary arterial pressure and lowered the relative medial thickness (RMT) and relative medial areas (RMA).⁶ H₂S also reduced inflammation in the pulmonary vasculature by reducing the intracellular cell adhesion molecule-1 (ICAM-1) in the pulmonary vasculature.⁶

We are aware of the potential limitations of this study. One of the major limitations was the use of Doppler echocardiography to measure the sPAP instead of right heart catheterization (RHC) although DE is a common measure to estimate the sPAP and has high correlation with the measurement done by RHC.¹⁷ DE has been shown to have a lesser precision

in estimating sPAP in compared to RHC in certain clinical conditions such as in advanced pulmonary diseases. However, RHC is an invasive procedure with several fatal complications²⁷ and in advanced-stage COPD with acute exacerbation and/or hypertension in which the patients become hemodynamically unstable, such invasive diagnostic procedures may impart severe adverse health effects. Furthermore, in countries like India where there is a lack of consensus guideline of medical procedures, add-on tests are often disregarded by the patients' families primarily because of the costs of the procedure as most of the individuals are not benefited by social security system or health insurance policies. However, to overcome the potential procedural limitation and to nullify any suspected case of over or underestimation, each DE result was scrutinized by two blinded independent experts and the scores of sPAP obtained from them were tallied using Cohen's kappa for an inter-rater agreement and found a very high agreement ($\kappa = 0.93$, $p < 0.001$) between the observations. Medication can also alter the H₂S level in acute exacerbated COPD patients. Although the effects of COPD medications such as cholinergic antagonists and β -adrenergic agonists on endogenous H₂S is unclear, corticosteroids might impair the activity of H₂S synthesizing enzyme cystathionine γ -lyase (CSE), thus attenuating the H₂S production.^{28,29} In our study inhaled corticosteroids (ICS) were prescribed to the AECOPD patients as per the GOLD guidelines, therefore the effects of ICS on reduced H₂S cannot be ignored.

Although exposure to H₂S may lead to deplorable health consequences such as cancer, septic shock or acute pancreatitis, exogenous administration of H₂S donors and/or revival of the endogenous H₂S synthesis is now being considered as possible therapeutic approaches in treating several conditions.³⁰⁻³⁴ Studies on animal models and isolated human lung segments have demonstrated that H₂S donors ameliorates oxidative stress, airway inflammation, and remodeling³⁵ and provides protection against emphysema and pulmonary hypertension.^{25,35} However, therapeutic potential, efficacy and safety of exogenously administered H₂S-donors or H₂S augmenters in human systems are yet to be validated through clinical studies.

Study limitations

H₂S has been assayed with crude form of chromogen in spectrophotometer, this study can be done by mass spectrometry if it would have been available. Another limitation is use of non-invasive color Doppler echocardiography to measure sPAP instead of right heart catheterization, an invasive procedure. The study was done on a fewer number of subjects due to inadequate funds and time constraints. Some medications like corticosteroids may have interference with the H₂S synthesizing enzymes which may have affected the levels of serum H₂S. A patient registry and tracking system is lacking in most of the health-care settings in India, therefore, the history, course and progression of the disease remain undetermined in most of the cases. Finally, we could not perform high-resolution CT scans of all the patients which could be a useful instrument to quantify and grade the structural changes of the lungs. It was a pilot project to be followed up by a more detailed study recruiting more cases and controls.

CONCLUSIONS

The measurement of serum H₂S could serve as an important prognostic marker in COPD. A reduced level of endogenous H₂S may indicate the onset of an exacerbation and also the probability of PAH. However, more clinical studies are required to test the clinical utility and validity of serum H₂S as a diagnostic marker in COPD.

ABBREVIATIONS

AECOPD: Acute Exacerbation of Chronic Obstructive Pulmonary Disease, BMI: Body Mass Index, CBS: Cystathionine beta synthase, COPD: Chronic Obstructive Pulmonary Disease, CSE: Cystathionine gamma lyase, DE: Doppler Echocardiography, FeCl₃: Ferric chloride, FEV1: Forced Expiratory Volume, FVC: Fixed Vital Capacity, GOLD: Global Initiatives for Obstructive Lung Disease, H₂S: Hydrogen Sulfide, ICAM-1: Intracellular Cell Adhesion Molecule-1, NaHS: Sodium hydrogen sulfide, PAH: Pulmonary Arterial Hypertension, PBS: Phosphate buffered saline, PH: Pulmonary Hypertension, *Pa*CO₂: Partial pressure of Carbon dioxide, *Pa*O₂: Partial Pressure of Oxygen, RAP: Right Atrial Pressure, RHC: right heart catheterization, RMA: relative medial areas, RM: relative medial thickness, ROC: Receiver Operating Characteristic, sPAP: Systolic Pulmonary Arterial Pressure, SCOPD: Stable Chronic Obstructive Pulmonary Disease, SD: Standard Deviation, sRVP: Right ventricular systolic pressure, TR:Tricuspid regurgitant, UV: Ultra Violet.

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DECLARATIONS

Authors' contributions

Conceptualization: UKB, SS, SS, PS. Data curation: SS, PS, SS. Formal analysis: UKB, SS, PS, SS. Funding acquisition: N/A. Investigation: PS, SS, SS. Methodology: PS, SS, SS. Project administration: SS, UKB, SS, PS, SS. Resources: SS, SS, PS. Software: SB, UKB, SS. Supervision: UKB, SS, SS. Validation: SS, SS, UKB. Visualization: SS, UKB, SS. Writing-original draft: SB, SS, PS. Writing-review & editing: SS, SB, SS, PS.

Conflict of interest

None.

Data availability

The data that support the findings of this study are available from the corresponding author, SB, upon reasonable request.

Ethical approvals

The study was ethically approved by the Institutional Ethics Committee of Nilratan Sircar Medical College & Hospital, Kolkata, India (No. NMC-113), and all patients provided signed informed consent before participation.

Funding resources

No external funding was received.

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