EFFECTS OF ENDOTRACHEAL INTUBATION AND VENTILATION IN OBSTRUCTIVE LUNG DISORDER PATIENTS UNDERGOING SURGERY UNDER GENERAL ANAESTHESIA USING NMR SPECTROSCOPY STUDY OF LUNG METABOLITES

INTRODUCTION
Chronic obstructive pulmonary disease (COPD) is one of the leading cause of adult respiratory disease in the globe.(Rabe et al., 2007) COPD patients planned for surgeries under general anaesthesia can have exacerbations of airway inflammation. There is a need to identify the pathological changes associated with intubation and ventilation in COPD patients which can guide in the anaeasthetic management of the patient and may prevent morbidity associated with endotracheal intubation and ventilation. Nuclear magnetic resonance (NMR) spectroscopic analysis is a novel method in medical research to look at the interaction of general anaesthesia and pulmonary morbidity. 

NMR spectroscopy is a new reproducible and conclusive modality for prompt and convenient analysis of biological fluids. 1D and 2D spectral analysis of mini BAL fluid revealed chemical shifts of various molecules. Principal component analysis (PCA) loading plot did not show changes in miniBAL metabolites of COPD patients in a duration of 3 to 5 hours.

METHODS
This study was conducted in the Centre of Biomedical Magnetic Resonance, Lucknow (CBMR), INDIA and Sanjay Gandhi Post Graduate Institute of Medical Science (SGPGIMS), Lucknow, INDIA. The study was resumed after obtaining approval from the institutes ethical committee (IEC code no 2015-77-MD-85 ) and informed patient consents were taken before sample collections. ASA II patients, aged more than 18 years with COPD who were scheduled for various surgeries under general anaesthesia with IPPV by endotracheal tube were enrolled in the study. The criteria used for diagnosing COPD was based on clinical and GOLD (Global initiative for chronic lung disease) spirometric criteria. The severity of airflow obstruction was classified according to GOLD criteria as mild, moderate, severe, or very severe if FEV1 was 80, 50–80, 30–49 %, or <30 % of the predicted value respectively. All the COPD patients were advised to continue their bronchodilator therapy till the time of surgery and then in the post-operative period as scheduled. Recent infections were ruled out in every case to avoid any superimposed inflammatory process. Duration of surgery was recorded which was defined as the time period between the incision and wound closure. All the patients were perioperatively managed in a similar way. Premedications, intraoperative anaesthetic drug usages and extubation protocols were kept similar in order to negate the differences of drugs on the airway inflammation. No bronchodilators therapy were needed in any of cases intraoperatively. Samples were collected using mini bronchoalveolar lavage (mBAL) technique using "catheter in catheter" (described by Kapil et al 2011) five minutes “after intubation” and half an hour “before extubation.” The samples collected were sent for NMR spectroscopic analysis.

PROCESSING OF SAMPLES
NMR spectroscopy: 200 µL of a buffer solution (0.1 M Na3HPO4/NaH2PO4, pH 7.0) and 350 µL of BALF sample were mixed for NMR experiments to reduce the variation in pH. Bruker 800-MHz NMR spectrometer with a triple-resonance (1H, 13C, 15N, and 2H lock) cryogenic probe was used to collect all spectra.

All 1D 1H NMR (noiseyr1Din Bruker library) spectra with water suppression were measured using the spectral width of 20 ppm and a relaxation delay of 5 seconds 128 scans, 64 K data points. Internal calibration of chemical shifts was done to the TSP (Trimethylsilylproionate) peak at 0.0 ppm All 1D spectra were baseline corrected and manually phased and processed with a line broadening of 0.3 Hz. Two-dimensional 2D homonuclear and heteronuclear spectra were recorded for the NMR peak assignment purpose.

NMR SPECTROSCOPY AND STATISTICAL ANALYSIS
Different protons in a molecule resonate at different frequencies depending on the local chemical environment. The spectral data set is obtained from the detection and quantifications of the various resonating molecule. The vertical axis is signal strength (in arbitrary units) and the horizontal axis is NMR frequency (or chemical shift in parts per million (ppm)). Each molecule originates different peaks in the spectrum based on their unique chemical structure and biochemical groups (CH3, CH2, OH, etc.). These peaks can show multiplicity which denotes the number of peaks.

Demographic data were analyzed using SPSS version 22. A total of 40 spectra corresponding to 20 COPD patients who underwent elective surgery under general anaesthesia were subjected to multivariate analysis.
Multivariate projection methods like principal component analysis (PCA) provides information on differences and similarities between the metabolic pathways. The spectral profile or loading profile in the data describes the first principal component (PC1). It describes most of the variations in the data set. The second best profile describing the variation is the second principal component (PC2) which is orthogonal to the PC1. The principal components are composed of scores and loadings. The scores contain information about sample classes and loadings hold information about the variables (chemical shifts) in the data set.

RESULTS

Twenty patients were enrolled in the study. Seventy-five per cent (n = 15) of patients were male and twenty-five per cent (n = 5) of patients were female. Mean age of patients was 54.35 ± 12.33 years. COPD grading was done according to GOLD protocol. COPD grade II (70%) and COPD grade III (30%) were present. No COPD grade I and grade IV patients were present among our study group of 20 patients. All the patients were on long term bronchodilator therapy which included formoterol (n=6), salmeterol (n=5), budesonide (n=9). Five patients had a history of diabetes mellitus. Systemic inflammation, free radical injury and hyperglycemia can alter the natural history of COPD. All these patients were optimized for glycemic control before surgery and hyperglycemia were avoided perioperatively. We did an analysis of 40 mini BAL fluid samples. Serial and stepwise processing of the samples by NMR did not reveal any significant change in the metabolomics profile in between “after intubation” and “before extubation” of COPD patients which is consistent with the previous studies. Betaine and choline intensities were also found in the 1D spectra, conversion of betaine to choline can be due to the action of bacterial enzymes which might play a role in the metabolic pathways. The spectral profile or loading profile in the data set describes the first principal component (PC1) separated the sample and accounted for 24% of the variance. There was a significant superimposition of the two groups of samples, and no separation between these two groups was found in the first and second principal components (PC1 and PC2) were calculated for the models of comparing two groups of “after intubation” and “before extubation” mini BAL fluid samples.

DISCUSSION

COPD patients undergoing invasive interventions like endotracheal intubation has a likelihood of predisposition to the enhancement of local and systemic inflammation due to multiple factors. Surgery and anaesthetic agents can have variable effects on the respiratory system of these patients. Patients factors like duration, severity, the grade of disease and treatment profile can have an influence on the degree of inflammation of airways. External factors like anaesthetics drugs, type and duration of surgery are equally important in the process of airway changes. Metabolomics studies have been done previously in obstructive lung patients using various specimens like serum, urine, saliva and exhaled breath condensate. In a study of metabolomics applied to exhaled breath condensate in childhood asthma, peaks in 3.0 to 3.4 ppm range of NMR spectra was observed representing oxidative compounds. Our study also showed a peak in the range of 3 to 4 ppm in 1 D spectral analysis and suggest the oxidative stress in COPD patients. In another study by de la rente and co-workers, pyruvate, succinate and choline in mini BAL of COPD patients but glutamine was absent. These differences show the diversity of metabolomics profile of different specimen of COPD patients. Intensities of branched-chain amino acids like leucine and isoleucine can be an indicator of increased muscle breakdown in COPD patients which is consistent with the previous studies. Betaine and choline intensities were also found in the 1D spectra, conversion of choline to betaine can be due to the action of bacterial enzymes which suggest features of bronchitis in COPD. Taurine intensities signify the role of smooth muscle relaxation in COPD and airway neutrophilia and...
oxidative stress. Although the above-mentioned metabolites signify pathophysiology of COPD patients compared to previous studies; no changes in the metabolomics profile of airway was noted during surgery of COPD patients in our study.

In a previous study by Singh et al. number of metabolites were found to be increased in cases of ALUARDS in intensive care units. The predominant metabolites which showed elevated concentrations were lactate, branched-chain amino acids, threonine and nucleotides degradation products. Increase in these metabolites was explained by the acute nature of acute lung injury and severe oxidative stress. But our patients were having chronic lung pathology and were not in any acute exacerbations. In our study, serial and stepwise processing of the samples by NMR did not reveal any significant change in the metabolomics profile in between “after intubation” and “before extubation” group of samples. It was concluded that COPD grade II and grade III patients do not show any change in inflammation of airways by general anaesthesia during surgery of 3 to 5 hours. It can be deduced from this study that the severity of inflammation caused by endotracheal intubation and ventilation in a chronically obstructed inflamed airway is not exaggerated during surgery. Our study had certain limitations. All the patients were in GOLD COPD grade II and grade III category. These patients were well optimized before elective surgery and were not in acute exacerbations at the time of sample collection. Well-optimized patients before surgery may have a lesser degree of airway inflammation compared to an unoptimized or acutely exacerbated reactions of COPD patients. No changes in metabolomics during intubation and ventilation of these patients can be due to control of airway inflammation and decrease in reactivity due to long term therapy. Patients who are in acute exacerbation can be expected to have different metabolomics profile and further studies are needed to explore the metabolomics profile of such patients by the same techniques.

REFERENCES


