Original Article

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# Analysis of Exhaled Breath Condensate pH in Asthma among Indian Population: A Pilot Study

Internal Medicine Section

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## ABSTRACT

**Introduction:** Asthma is an inflammatory disorder of lungs and analysis of Exhaled Breath Condensate (EBC) pH may give insights on control of asthma. To the best of our knowledge, this is the first study of EBC pH among Indian population.

**Aim:** To study EBC pH among asthmatics in Indian population.

**Materials and Methods:** In this cross-sectional study, EBC pH was compared with the control of asthma as well as severity of obstruction. There were 17 males and 33 females of bronchial asthma without exacerbation in a tertiary care hospital of a metro city. EBC pH was measured without deaeration using the Arterial Blood Gas (ABG) analyser and a pH electrode. Severity of asthma was defined as per Global Initiative of Asthma (GINA) guidelines into mild, moderate and severe based on treatment required to maintain control over asthma.

**Results:** Mean age of participants was 36 years. Mean EBC pH of the study participants was 7.341. Mean EBC pH in males was 7.391. Mean EBC pH in females was 7.316. Mean EBC pH among well controlled asthmatics was 7.330. Mean EBC pH among partially controlled asthmatics was 7.354. There was no significant correlation between EBC pH in well controlled and partially controlled asthmatics (p=0.71). Among subjects with normal spirometry, mean EBC pH was 7.34. Mean EBC pH among patients with moderate obstruction was 7.30. There was a consistent difference in pH of EBC when measured by a pH electrode as compared to ABG machine. Samples measured by pH electrode were acidic by pH difference of 0.8.

**Conclusion:** We found no correlation of EBC pH with asthma control. Method used for testing pH of EBC affects the result and has to be considered while interpreting studies.

**Keywords:** Bronchial asthma, Breath analysis, Spirometry

## INTRODUCTION

Bronchial asthma is defined as a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation [1].

EBC analysis is a method of condensing the exhaled air into liquid by exposing it to a cold surface in an EBC collecting device. This liquid is a matrix of biomarkers like other body fluids [2]. This liquid is then analysed for its individual constituents as per requirement.

EBC pH is a non-invasive, effort independent way of monitoring airway inflammation and simple assay that can be performed repeatedly without adversely affecting a patient, and can be collected safely both orally and from endotracheal and tracheostomy tubes. EBC pH measures remain the most successful manner to date to evaluate the role of airway acidification in respiratory disease [3]. Measurement of pH in EBC is very reproducible, relatively easy to perform, and provides almost real-time results [4].

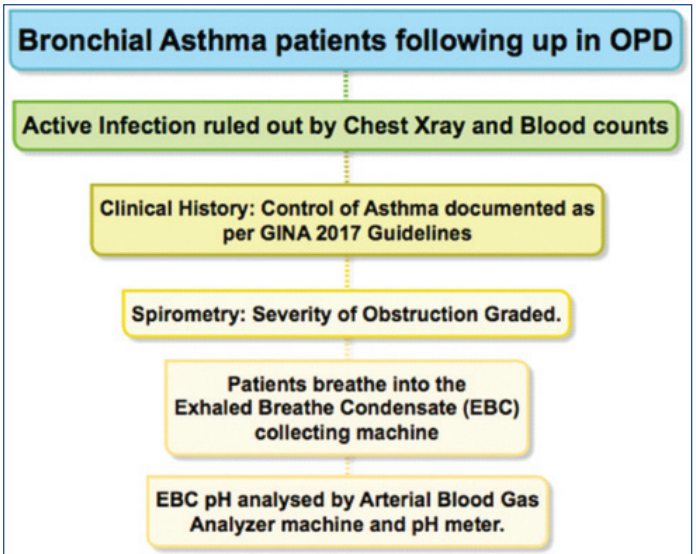
By assessing the EBC pH and correlating with the control of asthma and severity of obstruction we tried to find out if this method can be used to gauge the control and severity of asthma. Previous studies have reported conflicting results on this subject, and no study has been done in Indian population.

Also, if there is a correlation of EBC pH and control of asthma, it can be used to titrate the use of anti-inflammatory medications. This being a non-effort dependent method, has the potential of contributing to asthma management.

## MATERIALS AND METHODS

This was a cross-sectional study on 50 bronchial asthma patients following up in OPD of a tertiary care hospital in a metro city of India. This study was conducted from September 2013 to September 2015. An approval by Ethics committee was taken (EC/78/2014). Patient verbal consent was obtained. Sample size was limited to 50 as this was a pilot study. Non-smoking population between 12-70 years of age were included in this study. Active infection was ruled out by blood haemogram and chest radiograph. Asthma control was documented as per GINA 2015 guidelines into well controlled and partially controlled. Uncontrolled asthma was excluded from the study.

After appropriate instructions patients underwent EBC collection using EcoScreen. EBC collection method involved patients rinsing their mouth and breathing at total volume in the mouth piece of EcoScreen for approximately 10 minutes, thus giving us approximately 3 mL of exhaled breath fluid for analysis. This fluid was analysed by ABG analyser machine. Initial 20 samples were simultaneously analysed on ABG analyser machine and pH electrode {ISFET electrode (Sentron, the Netherlands)} [Table/Fig-1]. Samples were analysed within 5 mins of collection. No deaeration was done. After collection of EBC, patients were made to perform spirometry. As per 2015 Global Initiative for Obstructive Lung Diseases guidelines obstruction on spirometry was documented when FEV1/FVC <0.70. Severity of obstruction based on FEV1 (% of predicted) was as follows, Mild 80-100%, Moderate 50-80%, Severe 30-50%, Very Severe <30% (GOLD 2015).




**[Table/Fig-1]:** Methodology of the study.

Data so obtained was analysed using one-way ANOVA and regression analysis.

## RESULTS

In our study involving 50 bronchial asthma patients, 17 were males and 33 females. A pH values were reported as recorded by ABG analyser. Mean EBC pH of the participants was 7.341.

Mean EBC pH in males and females was 7.391 and 7.316 respectively. There was no significant association between gender and EBC pH (p=0.13).

Age of the study population ranged from 13 to 66 years (mean: 36.52). There was no correlation between age and EBC pH (r=-0.08, p=0.54) [Table/Fig-2].

| Gender | Number | EBC pH Minimum | EBC pH Maximum | EBC pH Mean | Standard Deviation |
|---|---|---|---|---|---|
| Males | 17 (34%) | 7.12 | 7.64 | 7.391 | 0.171 |
| Females | 33 (66%) | 7.05 | 7.65 | 7.316 | 0.162 |

**[Table/Fig-2]:** Gender and EBC pH.

Asthma symptoms of 34 were well controlled and 16 patients were partially controlled. Mean EBC pH of the study in asthmatics was 7.341. Mean EBC pH among well controlled asthmatics was 7.330. Mean EBC pH among partially controlled asthmatics was 7.354. There was no significant correlation between EBC pH in well controlled and partially controlled asthmatics (p=0.71) [Table/Fig-3].

| Asthma Control | Number | EBC pH Minimum | EBC pH Maximum | EBC pH Mean | Standard Deviation |
|---|---|---|---|---|---|
| Well Controlled | 34 (68%) | 7.05 | 7.65 | 7.33 | 0.181 |
| Partially Controlled | 16 (32%) | 7.12 | 7.63 | 7.354 | 0.149 |

**[Table/Fig-3]:** Asthma control and EBC pH.

A total of 37 (74%) patients had normal spirometry, 2 (4%) had mild obstruction, 8 (16%) had moderate obstruction, 2 (4%) had severe obstruction, 1 (2%) had very severe obstruction. Among subjects with normal spirometry, mean EBC pH was 7.34. Mean EBC pH among patients with moderate obstruction was 7.30. There was no significant association between severity of obstruction and EBC pH (p=0.85) [Table/Fig-4].

There was a consistent difference in pH of EBC when measured by a pH electrode as compared to ABG machine. Samples measured by pH electrode were acidic by pH difference of 0.8.

| Spirometry | Number | EBC pH Minimum | EBC pH Maximum | EBC pH Mean | Standard Deviation |
|---|---|---|---|---|---|
| Normal | 37 (74%) | 7.05 | 7.65 | 7.34 | 0.167 |
| Mild Obstruction | 2 (4%) | 7.28 | 7.56 | 7.42 | 0.197 |
| Moderate Obstruction | 8 (16%) | 7.12 | 7.46 | 7.3 | 0.122 |
| Severe Obstruction | 2 (4%) | 7.2 | 7.34 | 7.27 | 0.09 |
| Very Severe Obstruction | 1 (2%) | NA | NA | 7.33 | NA |

**[Table/Fig-4]:** Severity of airway obstruction and EBC pH.

## DISCUSSION

Objective assessment of asthma control has a significance is modulating treatment. We rely on patients subjective reporting of complains and modify treatment accordingly. Often this leads to improper stepping up or stepping down of treatment. Hence there is a need of an objective parameter to gauge asthma control.

Exhaled nitric oxide (FeNO) is being often used for assessing asthma control and has been found to be significantly correlating to asthma control [5]. However, due to significant limitations of various studies assessing role of FeNO in asthma including small number of subjects and short-term follow up, routine assessment of FeNO in assessment of asthma has not been recommended [6].

EBC condensate level of leukotriene E4 is increased in asthma but is not detectable in normal subjects or in patients with Chronic Obstructive Pulmonary Disease (COPD) [7]. EBC level of hydrogen peroxide has also been shown to be a promising biomarker for assessment of asthma [8].

However, the above-mentioned assays for EBC analysis are expensive and in a resource limited setting like India, an economical assessment of asthma control is desired.

The inflammatory markers including Nitric Oxide, leukotriene E4 and hydrogen peroxide result in acidification of exhaled air. Studies in asthma have pointed to the role of airway acidification in the airway pathophysiology [9]. Acidity (pH) is the most easily measured and widely studied biomarker in EBC [10].

EBC can be collected by two of the commercially available devices, namely, portable RTube and non-portable EcoScreen. There is a poor correlation between EBC pH obtained by RTube and EcoScreen [11-13].

Normal values of gas-standardized ("$CO_2$-free") EBC pH have been reported from multiple investigators and range between 7.5 and 8.1 [14-25]. The difference between pH values of deaerated and non-deaerated samples collected by EcoScreen, on immediate analysis has been studied in 23 subjects (including 10 asthmatics). Mean pH has been reported as 6.99 (6.85–7.13) and 8.17 (8.08–8.26) respectively [12]. In comparison to the above studies the Mean EBC pH in our study was 7.341 (7.05–7.65, SD: 0.167).

Deaeration is a process of removing $CO_2$ from the EBC sample by bubbling Argon gas through the sample. Deaerated sample is known to have stable pH and a reproducible pH value after a year. The act of deaeration makes the sample less acidic by removing $CO_2$ [4]. All the samples we studied were non-deaerated and analysed immediately after collection and thus were comparable.

There have been conflicting results on association between airway obstruction and EBC pH [26,27]. Our study has shown no significant correlation with severity of airway obstruction and EBC pH.

Our study documented no correlation of EBC pH with age and sex is consistent with other studies done in healthy population [16].

In previous study done by Kostikas K et al., it was concluded that EBC pH may identify patients with not well controlled asthma, however its utility may be limited to certain selected subgroups [28]. Ratnawati et al., have studied EBC pH in paediatric population with asthma, atopy and normal children and have concluded that there was no difference in EBC pH between these 3 groups however asthmatics with FEV1 <80% predicted had significantly lower pH

[29]. We compared well controlled and poorly controlled asthma and have not found any significant correlation. It is worth a mention that we have not studied EBC pH in uncontrolled asthma.

We were not able to explain the differences in pH measurements between ABG analyser machine and pH electrode. But on a similar context with pleural fluid analysis this was consistent with the recommendation of using blood gas analyser for assessing pleural fluid pH over use of pH electrode [30,31].

## CONCLUSION

We found no correlation of EBC pH with age, sex, severity of obstruction and control of asthma among patients with well controlled and partially controlled asthmatics. Method used for testing pH of EBC affects the result and has to be considered while interpreting studies. Further studies will be needed in normal Indian subjects and those with uncontrolled asthma to know efficacy of EBC pH in documenting objective control of asthma.

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