ABSTRACT
Background: The measurement of ECO may represent a new method for the non-invasive monitoring of airway inflammation and oxidant stress in Chronic Obstructive Pulmonary Disease, asthma, bronchiectasis, cystic fibrosis patients. Quantification of lung oxidative stress in stable COPD patients by measuring ECO levels may also contribute to the understanding of the pathophysiology of COPD.
Aims & Objective: To study the utility of measuring Exhaled Carbon Monoxide (ECO) level in addition to Pulmonary Function Test (Spirometry) in the monitoring of Chronic Obstructive Pulmonary Disease (COPD).
Materials and Methods: COPD patients who were smokers and with a history of exposure to wood smoke (n =60) and healthy non-smokers as control (n =40) were selected as subjects by fulfilling the exclusion criteria as per the GOLD guidelines. Clinical examinations and spirometry including reversibility test were made following the standard protocol/procedure. ECO was measured using a MICRO III Smokerlyser.
Results: The difference in level of ECO between COPD cases and healthy non-smokers was highly significant (F = 23.897; df = 98; p < 0.0001). The difference in the level of ECO among different groups (mild, moderate, severe and very severe) was highly significant (F=15.995; df =2; p<0.0001). ECO level in female COPD cases who were exposed to wood smoke was elevated (4.11 ±0.0001). The difference in the level of ECO among different groups (mild, moderate, severe and very severe) was highly significant (F =1.593; df =30; p < 0.0001).
Conclusion: ECO levels in COPD cases vary with different grades of airway obstruction. We concluded that measuring the level of ECO in COPD cases along with spirometry forms a new approach for better understanding of pathophysiology of COPD cases, with indirect assessment of airway inflammation, oxidative stress and severity of airway obstruction.
Key Words: Air Way Inflammation; Air Way Obstruction; Chronic Obstructive Pulmonary Disease (COPD); Exhaled Carbon Monoxide (ECO); Cough, Smoking Index; Dyspnoea; Oxidative Stress

Introduction
Chronic Obstructive Pulmonary Disease (COPD) is a disease of increasing public health importance around the world. Cigarette smoking is the notorious and the commonest cause of COPD. Hereditary deficiency of alpha-1 anti-trypsin is one of the best documented genetic risk factors. Besides all these, many inhalation exposures other than cigarette smoke including occupational dusts (irritants, vapours, fumes) and exposure to biomass fuel in confined spaces are known to cause COPD on their own. The impact of COPD on an individual patient depends not just on the degree of airflow limitation, but also on the severity of symptoms (especially breathlessness and decreased exercise capacity). There is only an imperfect relationship between the degree of airflow limitation and the presence of symptoms. Spirometric staging, therefore, is a pragmatic approach aimed at practical implementation and should only be regarded as an educational tool and a general indication to the initial approach to management.

Lung function declines gradually over decades in the patients with COPD, but recognition of early stages of COPD is possible with prompt use of lung function testing, particularly spirometry. The forced expiratory spirogram is the most useful test of airflow dynamics. Yearly measurement for at least 3 years is required to assess the rate of decline in FEV1 (Forced expiratory volume in 1 sec), and rates> 50 ml/year suggest accelerate decline. Post-bronchodilator FEV1 is the mainstay of classification of severity of COPD and it is strongly predictive of subsequent mortality from COPD. There are, however, limitations in the use of this measurement, since changes in FEV1 over time are small in relation to repeatability of the measurement.

Oxidative stress (OS) is a major pathogenic component of airway inflammation that characterizes COPD. Oxidants decrease, the activity of elastase inhibitors and an imbalance between oxidants and antioxidants may play an important role in the pathophysiology of COPD. Plasma antioxidant capacity is decreased in the patients with acute exacerbations of COPD and returns toward normal values during treatment. OS is also increased in chronic healthy smokers.
The measurement of ECO may represent a new method for the non-invasive monitoring of airway inflammation and oxidant stress in COPD patients. Carbon monoxide (CO) is produced ubiquitously in the body by heme oxygenase (HO) as a breakdown product of heme.[12-15] HO has been found in the pulmonary vascular endothelium and in alveolar macrophages.[16-17] CO causes bronchodilatation in vivo and this finding suggests a role for endogenous CO in inflammatory airway diseases.[18] ECO has also been used to quantify oxidative stress in stable asthma and bronchiectasis patients who have higher CO levels than healthy subjects.[19,20] ECO has also been reported to increase in stable cystic fibrosis patients and, to a greater extent, during exacerbations.[21] Most of the studies linking COPD with oxidative stress were performed in vitro, using invasive techniques such as examination of BAL fluid or measurement of systemic rather than oxidant stress. Therefore a study was undertaken to quantify lung oxidative stress in stable COPD patients (current and ex-smokers) by measuring ECO levels. This may contribute to the understanding of the pathophysiology of COPD and may suggest a potential new non-invasive method to monitor airway inflammation in this disease. In addition to it, spirometry has also been done, and correlation of level of airway obstruction with level of ECO has also been made so as to assess the combined efficacy of measurement of ECO and spirometry.

Materials and Methods

COPD patients who were smokers (current and ex-smokers); COPD patients with history of exposure to wood smoke and healthy non-smokers were selected as subjects and the study was approved by the Ethical Committee. A total of 60 patients (n=60) stable COPD patients, were included in this study after fulfilling the exclusion criteria. Forty individuals (n=40) were taken as controls for the study.

Patients with typical symptoms of chronic cough with or without expectoration with shortness of breath on exertion were included in the study after confirming the diagnosis by FEV1/FVC < 70% and post broncho-dilator FEV1 < 80% on spirometry as per the GOLD guidelines. Clinical examinations were made following the standard protocol/procedure. Chest X ray, hematological and biochemical parameters and ECG with echocardiography were made. Spirometry including reversibility test was performed maintaining ATS recommendation on Elite Series Body Plethysmograph (Make- Medical Graphics Corporation, USA; Software- Breeze Suite Version 6.2C). The tests were carried out during 10.00-14.00 hours. Necessary instructions were given to patients prior to PFT. Reversibility testing was also performed. Post Bronchodilator FEV1 was recorded in all cases to assess severity of air way obstruction. Smoking Index was calculated by asking the subjects about the number of cigarettes or Bidi smoked by them in a year.

ECO Analysis: Breath CO monitoring was performed using a MICRO III Smokerlyser (Bedfont Instruments; Kent, UK). The subjects were asked to exhale completely, inhale fully, and then hold their breath for 15sec. If the subjects were unable to hold their breath for 15sec, they were asked to hold it for as long as possible by them. Following breath holding, the subjects were asked to exhale slowly into the Smokerlyser and were encouraged to exhale fully in order to sample the alveolar air.

Statistical Analysis: Chi-Squire test was used to test the hypothesis of association between duration of symptoms (dyspnoea, cough, wheezing), duration of wood smoke exposure and smoking index with severity of air way and ECO levels in COPD cases. Independent t-test was used to compare the mean percentage predicted from the spirometric parameters between COPD cases and healthy non-smokers. One way Analysis of Variance (ANOVA) was performed to compare the mean values in three groups of air way obstructions (mild, moderate, severe and very severe) among the COPD cases. Pearson bivariate correlation co efficient was used to quantify the extent of correlation between spirometric parameters with the ECO levels among COPD cases. The results are mentioned in mean ± standard deviation. For all statistical analysis, p<0.05 was considered as significant and p<0.0001 was considered as highly significant.

Results

Age group of COPD cases and healthy non-smokers were 41-60 years and above. Males constituted 70% among COPD cases and 62.5% among healthy non-smokers.

Dyspnoea

A significant association was observed between the duration of dyspnoea with severity of airway obstruction among the COPD cases ($X^2=19.680; \text{ df} = 9; \ p<0.05$). COPD patients with dyspnoea of duration 1-10 months, most of the cases (65%) (n=13) had ECO of 3-4ppm. In patients with dyspnoea of duration 11-20 months, 50%
(n=10) of them had ECO level > 4ppm and 40% (n=8) had ECO level of 3-4 ppm. In patients with dyspnoea of duration 21-30 months 57.1% (n=8) had ECO level > 4 ppm, and 42.9% (n=6) had level of 3-4ppm. Finally, in patients with dyspnoea >30 months duration, 66.7% (n=4) of them had ECO level of 3-4 ppm, and 33.3% (n=2) had ECO level >4ppm. However, no significant association was noticed between duration of dyspnoea and ECO level among COPD cases (X² = 5.46; df= 6; p=0.485).

### Cough

No significant association was demonstrated between duration of cough with severity of airway obstruction among the COPD cases (X² = 11.775; df = 6; p= 0.067). It was noted that 58.3% (n=7) of COPD cases with cough for 1-3 months had ECO level of 3-4ppm, 33.3% (n=4) had exhaled CO level >4ppm. 73.3% (n=11) of COPD cases with cough more than 3 months had ECO of 3-4ppm and 20% (n=3) of the cases had ECO level of >4ppm. Also no significant association was observed between duration of cough and level of ECO among the COPD cases(X² = 6.741; df=4; p=0.150).

### Wheezing

No significant association was demonstrated between duration of wheezing with severity of airway obstruction among the COPD cases(X² =9.876; df = 6; p=0.130). Among COPD patients who had wheezing of 1-3 months, 63.6% (n=6) of them had ECO level of 3-4 ppm, 36.4% (n=4) had level of >4ppm and in patients with wheezing of duration >3months, 75% (n=12) had ECO level of 3-4ppm and 18.8% (n=3) of them had ECO level of >4ppm. Further, there was no significant association between duration of wheezing and ECO level among the COPD cases (X² = 8.061; df=4; p=0.089).

### Wood smoke

COPD females who have been exposed to wood smoke for 10 to 20 years and >20 years suffered moderate and severe airway obstructions respectively. But there was no significant association between duration of wood smoke exposure and severity of airway obstruction among the COPD cases (X² = 6.993; df= 6; p=0.322). Though ECO got elevated among smoke exposed COPD cases, there was no significant association between duration of wood smoke exposure and ECO level (X²= 5.280; df= 4; p=0.260) (Table 1 & 2).

### Table 1: Distribution of ECO level and severity of airway obstruction in COPD patients

<table>
<thead>
<tr>
<th>Severity of Airway Obstruction</th>
<th>≤ 2 ppm</th>
<th>3-4 ppm</th>
<th>&gt; 4 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>0</td>
<td>3 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>3 (16.7%)</td>
<td>15 (83.3%)</td>
<td>0</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>9 (47.4%)</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td>Very severe</td>
<td>0</td>
<td>4 (20%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3 (5%)</td>
<td>31 (51.7%)</td>
<td>26 (43.3%)</td>
</tr>
</tbody>
</table>

### Table 2: Association of ECO level and severity of airway obstruction in COPD patients

<table>
<thead>
<tr>
<th>Value</th>
<th>df</th>
<th>Asymp. Sig. (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>31.484(a)</td>
<td>.000</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>39.878</td>
<td>.000</td>
</tr>
<tr>
<td><strong>No. of Valid Cases</strong></td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Distribution of smoking index and ECO level in COPD patients

<table>
<thead>
<tr>
<th>Smoking Index</th>
<th>≤ 2 ppm</th>
<th>3-4 ppm</th>
<th>&gt; 4 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 500</td>
<td>0</td>
<td>11 (47.8%)</td>
<td>12 (52.2%)</td>
</tr>
<tr>
<td>501-1000</td>
<td>1 (7.1%)</td>
<td>8 (57.1%)</td>
<td>5 (35.7%)</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>0</td>
<td>0</td>
<td>5 (100%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1 (4.7%)</td>
<td>19 (44.2%)</td>
<td>22 (51.2%)</td>
</tr>
</tbody>
</table>

### Table 4: Association of smoking index and ECO level in COPD patients

<table>
<thead>
<tr>
<th>Value</th>
<th>df</th>
<th>Asymp. Sig. (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>27.788(a)</td>
<td>.000</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>16.427</td>
<td>.012</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>2.330</td>
<td>.127</td>
</tr>
<tr>
<td><strong>N of Valid Cases</strong></td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Comparison of ECO between female COPD cases who are exposed to wood smoke and healthy female non smokers

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female COPD with wood smoke exposure</td>
<td>18</td>
<td>4.11</td>
<td>1.32</td>
<td>0.312</td>
</tr>
<tr>
<td>Healthy non-smokers females</td>
<td>14</td>
<td>1.50</td>
<td>0.519</td>
<td>0.139</td>
</tr>
</tbody>
</table>

### Smoking

Severity of airway obstruction increased with increase in smoking index among the COPD cases and this association was significant. (X²= 22.548; df= 9; p<0.05). There is an increase in the level of ECO with increase in smoking index among the COPD cases and this association was highly significant(X²= 27.788; df= 6; p<0.0001). ECO level increased with increase in severity of airway obstruction among the COPD cases (Table 3 & 4) and this association was highly significant (X²= 31.484, df=6, p<0.0001). Spirometric parameters with respect to healthy non-smokers and COPD cases are presented in Table 3. There was a highly significant difference in pre bronchodilator (baseline) percentage predicted levels of spirometric parameters between COPD cases and healthy non-smokers. (p<0.0001).

Highly significant difference was noted in ECO level between COPD cases and healthy non-smokers.
Further, there was a significant negative correlation of FEV1 % with ECO level \( (r = -0.635, p < 0.01) \) among the COPD cases. Thus, when there was decline in FEV1 %, there was an increase in the level of ECO among COPD cases. There was also a significant negative correlation of FVC% and FEF25-75% with the level of ECO and so ECO level increased with decrease in % FVC and %FEF25-75% \( (r = -0.436, p < 0.0001) \).

ECO level in female COPD cases who were exposed to wood smoke was elevated when compared to healthy female non-smokers and the difference was highly significant. \( (F=1.593; df=30; <0.0001) \) (Table 5).

**Discussion**

The prevalence of COPD is more among males than females.[22-24] In a study conducted by Jindal et al, in India, most of the COPD cases were bidi smokers and all of them were males.[23] Majority of the COPD cases have been reported to have moderate (51.4%) and mild (30.6%) airflow obstruction.[24] But in our study, the majority of COPD cases had severe (33.3%) and very severe (31.7%) airway obstruction and this reflected the result of the earlier study conducted in South India.[27] The severity of COPD among our study subjects might be due to lack of awareness of the symptoms of COPD and the patients usually present late in tertiary clinic.

We found that the severity of airway obstruction increased with increase in duration of dyspnoea among the COPD cases. Due to reduced airway caliber and increased airway resistance there is reduced airflow particularly during expiration which prolongs the removal of air from the lungs.[28] It has been reported that cough reflex sensitivity is heightened in COPD patients compared to healthy volunteers and similar to that of asthma patients.[29] The degree of airway obstruction does not predict cough reflex sensitivity or objective cough counts, implying an independent process and therefore there was no significant relation between cough and degree of airway obstruction among the COPD cases in our study. Further, there was no significant association observed between duration of wheezing with severity of airway obstruction among the COPD cases \( (X^2=12.406, df= 8, p=0.130) \).

Female COPD cases usually have the history of exposure to wood smoke as they involve in household works particularly cooking by burning wood, biomass etc. Duration of exposure may be related to the degree of airway obstruction and the association between length of exposure and COPD suggests a dose-response pattern.[30] However, no such relationship was observed in the present study.

When there is increase in smoking index, there would be significant increase in the severity of obstruction in spirometry as evidenced from our study \( (X^2 = 22.548; df = 9; p<0.0001) \). This finding is similar to the observation of Cheng et al. and they found that higher the smoking index, the higher the incidence of COPD and smoking was found to be the major causative factor.[31] Pulmonary Function Test using spirometry conducted in a rural area in India among smokers and non-smokers revealed that bidi smoking is the most common cause of obstructive lung changes in smokers.[32]

The level of ECO increased with increase in smoking index among the COPD cases and the association was highly significant \( (X^2 =27.788; df =6; p <0.0001) \). In our study, the elevated level of ECO among smokers with COPD \( (4.55 \pm 1.443) \) when compared to healthy non-smokers \( (1.48 \pm 0.506) \) corroborates with the finding of Edward Middleton et al.[33] Significantly higher level of ECO among bidi smokers when compared to cigarette smokers in our study is similar to the finding of a study conducted in India.[34] In our study, ECO level was measured in smokers with COPD whereas in the former study it was measured in smokers in general and this might be the reason for low average level of ECO. Average level of ECO among COPD patients was significantly elevated when compared to the level in healthy non-smokers \( (p <0.05) \).

One of the few studies which measured the level of CO and ECO among COPD cases reported that mean CO levels were higher in ex-smokers with COPD than in non-smoking controls but lower than in current smokers with COPD.[35] But the level of ECO among COPD cases in our study was low when compared to the finding of the above study. This difference in the level may be explained by the fact that there are many factors which affect the level of ECO like timing of performance, ability of the subject to hold the

Table 6: Levene’s Test for Equality of Variances

<table>
<thead>
<tr>
<th>Equality of Variances</th>
<th>Levene’s Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECO in ppm</td>
<td>Assumed</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.593</td>
</tr>
<tr>
<td></td>
<td>Not assumed</td>
<td>7.649</td>
</tr>
</tbody>
</table>

**Table 6: Levene’s Test for Equality of Variances**

(F=23.897; df=98; p<0.0001). Further, there was a significant negative correlation of FEV1 % with ECO level \( (r = -0.635, p < 0.01) \) among the COPD cases. Thus, when there was decline in FEV1 %, there was an increase in the level of ECO among COPD cases. There was also a significant negative correlation of FVC% and FEF25-75% with the level of ECO and so ECO level increased with decrease in % FVC and %FEF25-75% \( (r = -0.436, p < 0.0001) \).
breath for 15 seconds and exhale fully, geographic variation. In the above-referred study there was no correlation observed between ECO and lung function tests in any group of patients but in our study, there was a significant association of severity of airway obstruction with the level of ECO, i.e. ECO level increased with increase in severity of airway obstruction i.e. from mild grade to very severe grade among the COPD cases ($X^2$= 31.484; df= 6; p<0.0001).

In a study on the assessment of pro inflammatory cytokines and carbon monoxide in exhaled breath condensate of smokers, a positive correlation with daily consumption of number cigarettes and duration of smoking was found besides a significant negative correlation of ECO level with FEV1, FVC, IL-6.[4] The above finding is similar to our study, in which the level of ECO increased with increase in smoking index among the COPD cases which had highly significant association ($X^2$=27.788; df=6; p <0.0001)

In our study, it was found that ECO level was elevated in smokers with COPD than healthy non-smokers. In a similar observation reported elsewhere, it was found that the level of ECO in current smokers with COPD was higher than in healthy non-smokers and ex-smokers with COPD.[37]

In a study conducted in an arid high altitude of North India, it was noted that ECO was higher in smokers than in non-smokers. But in non-smoking men and the women who were exposed to fire smoke, levels of ECO were significantly higher in winter than in summer as ECO varies with seasonal variation and change in environment.[38] We also noted that ECO level increased in the COPD cases with history of smoking and wood smoke exposure. However, the duration of exposure to wood smoke among females did not have any significant relationship with the severity of air way obstruction.

We found that ECO levels in COPD cases vary with different grades of air way obstruction. It has been also shown that exhaled CO levels increases during an asthma and COPD exacerbation.[39-41] Also ECO could serve as an indicator of acute exacerbations in children with CLD.[42] It is likely that ECO derives from an endogenous source since the inhaled HO inhibitor, tinnmesoporphyrin, significantly inhibits the concentration of CO.[43] ECO measurement is simple, reproducible and non-invasive. Therefore, measuring the level of ECO in COPD cases along with spirometry forms a new approach for better understanding of pathophysiology of COPD cases, with indirect assessment of airway inflammation, oxidative stress and severity of airway obstruction.[44] ECO values can be used as potential indicators of inflammation in asthma, stable COPD and exacerbations, cystic fibrosis and lung cancer but the potential diagnostic value of ECO is yet to be completely characterized as a marker of inflammation.[45]

**Conclusion**

Our study concludes that quantification of ECO along with spirometry could be a better choice than spirometry alone in the diagnosing and management of COPD cases.

**References**


Yuvarajan Sivagnaname. Utility of measuring CO and spirometry

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