Breath Carbon Monoxide Concentration”, An Indicator of Early Airway Inflammation in Asymptomatic Smokers

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Abstract

Measurement of carbon monoxide(CO) concentration in expired air can be used for detecting and monitoring cytokine mediated inflammation and oxidative stress in the respiratory tract of smokers if smokers are made to abstain from smoking for 8 hrs. Total 48 apparently healthy male volunteers (24 were asymptomatic male tobacco smokers and rest 24 were healthy non-smoker males) with age between 18 – 25 years participated in the study. Exhaled CO level measured by the breath CO analyzer.

Baseline Pulmonary function test(PFT) was done using RMS Helios Series Computer based Spirometer. Exercise challenge test was done using treadmill and PFT was recorded immediately, 5min., 10min. and 20min. of recovery period. Comparison of lung function before and after exercise in healthy volunteers and asymptomatic smokers respectively was done using paired t test. Correlation between post exercise percentage change in FEV1 and exhaled CO was seen using Pearson’s coefficient of correlation. A negative correlation was found between CO concentration in exhaled breath and improvement in FEV1 after exercise challenge test. Hence, Breath CO analyser can be used to measure endogenous CO in so called “healthy smokers” as a tool to detect early inflammation and also to motivate them to quit smoking before the disease becomes irreversible.

Keywords – Carbon monoxide, Pulmonary Function test.

Introduction

Measurement of carbon monoxide concentration in expired air is used as an objective method to analyze the smoking status. It can also be used for detecting and monitoring cytokine mediated inflammation and oxidative stress in the respiratory tract of smokers if smokers are made to abstain from smoking for 8 hrs. Approximately 30% of smokers do not show chronic symptoms or abnormal lung function. Nevertheless, even these so-called “healthy smokers” show subtle changes in lung morphology, lung inflammation and lung function. Smoking, apparently, always affects the lungs, although the extent and severity of these changes differ between individuals. Airway response to exercise and endogenous production of CO measurement can help to mark these changes in early asymptomatic smokers.

Material and Method

The study was carried out in Department of Physiology, Baroda Medical College, Vadodara. Total 48 apparently healthy male volunteers with age between 18 – 25 years participated in the study. Out of which 24 were asymptomatic male tobacco smokers and rest 24 were healthy non-smoker males.

They consisted of volunteers, hospital workers or university students as cases and healthy non-smoker males. Personal information was collected through questionnaire. Subjects were excluded from the study if there was any history of exercise discomfort, wheezing, taking any medications that might influence airway tone,
current cough, dyspnoea, sputum production, asthma, allergic rhinitis, hay fever, urticaria, other allergic conditions, or any respiratory infection within two months, cardiac disease, chest deformity, or occupational exposure to hazardous substances. Written and informed consent was taken from each and every participant. The participants were instructed to avoid heavy physical activities, abstain from tobacco in any form, alcohol for at least 12 hours and tea or coffee for 2 hours before coming to the research lab and the subject was advised to come two hours after light breakfast.

Each participant had 2 visits, first visit for general instruction about how to perform the test, so that they become accustomed with proper maneuver. The second visit involved the baseline and post exercise breathing tests. The experiment was done in morning hours 9:00 AM to 12:00 noon to avoid circadian variations in the pulmonary function. Participants were familiarized with the surroundings. Instructions for the whole procedure were given. All the subjects first expired in Breath CO analyzer to get the value of carbon monoxide in exhaled air (in ppm). Exhaled CO and %COHb was measured on a portable smokerlyzer (Breath CO monitor, Bedfont Scientific Ltd., Kent, England). In this procedure, participants were said to inhale deeply and hold their breath fully for 15 sec before exhaling into a disposable mouthpiece. The subjects exhaled slowly from total lung capacity with a constant flow. This procedure was repeated three times with 1 min of normal breathing between each repetition and the mean value was used for analysis. Exhaled CO level measured by the analyzer and was reported to correlate closely with blood COHb concentration. 

**Baseline PFT:** Lung functions were measured by RMS Helios Series Computer based Spirometer with highly advanced and user-friendly software offering 34 parameter readings, Pre-Post bronchodilatation results, Percentage Improvement & Lung Age Calculations. After giving rest for 15 minutes, baseline pulmonary function test was done using the FVC manoeuvre. Indices recorded were - Forced vital capacity (FVC), Forced expiratory volume in 1 second (FEV1), Mean forced expiratory flow between 25% and 75% of FVC (FEF25-75%), Mean forced expiratory flow between 75% and 85% of FVC (FEF75-85%). For baseline lung function three satisfactory manoeuvres were taken and best of three was chosen as final result.

**Exercise challenge testing:** For exercise challenge treadmill attached to 12 lead ECG monitor was used which continuously monitored heart rate and ECG while the subject was exercising. Participants were asked to exercise on the treadmill, so that they achieve 80-90% of the maximum predicted heart rate (220 - age) for 5 minutes. PFT was recorded immediately after completion of the exercise challenge. Other subsequent recordings were done at 5, 10 and 20 minutes of recovery period. Whilst several protocols exist, the most widely accepted protocol is based on the Guidelines produced by the ATS in 1999. The guiding principle is to create a degree of hyperventilation by pushing the participants quickly to perform high cardiac output exercise. This was done on the treadmill with the aim of achieving 80 – 90% of the maximal heart rate in 4 – 6 minutes. The heart rate was monitored through ECG and the test was stopped when the patient achieved 80 – 90% of the maximal cardiac output.

**Data entry and Analysis:** Data was entered in MS excel and analyzed using software MedcalC. Comparison of lung function before and after exercise in healthy volunteers and asymptomatic smokers respectively was done using paired t test. Correlation between post exercise percentage change in FEV1 and exhaled CO was seen using Pearson’s coefficient of correlation.

**Result**

There was no significant difference in age, height, weight and baseline pulmonary function among the 48 volunteers who participated in the study (Table1-2). They were divided into two groups, where the first group consisted of 24 healthy volunteers who had never smoked and the second group of 24 asymptomatic smokers who were smoking for not more than 5 years and none had score ND score (Nicotine Dependence) more than 5.

In our study we found negative correlation between exhaled CO concentration and FEV1 % change after exercise challenge when values of all the subjects in the study were compared irrespective of their smoking status. Fig 2 demonstrates the correlation between exhaled CO and FEV1 % change after exercise challenge in the participants (r=-0.45, p=0.001). The exhaled CO concentration was greater in the smokers as compared to the healthy subjects Fig 1(3.63±2.50 Vs 0.92±0.78) but the difference was not significant.
Table 1: General characteristics of the study group:

<table>
<thead>
<tr>
<th></th>
<th>Healthy Volunteers</th>
<th>Asymptomatic Smokers</th>
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</thead>
<tbody>
<tr>
<td><strong>Mean ±SD</strong></td>
<td><strong>Range</strong></td>
<td><strong>Mean ±SD</strong></td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.54 ±1.59</td>
<td>18 - 25</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.79 ±4.90</td>
<td>158 - 180</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.96 ±7.71</td>
<td>50 - 75</td>
</tr>
</tbody>
</table>

Table 2: Baseline Spirometry before exercise challenge in healthy volunteers and Asymptomatic Smokers:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Volunteers</th>
<th>Asymptomatic Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ±SD</strong></td>
<td><strong>Mean ±SD</strong></td>
<td></td>
</tr>
<tr>
<td>FVC (litres)</td>
<td>4.02 ±0.51</td>
<td>3.83 ±0.63</td>
</tr>
<tr>
<td>PEFR (lit/sec)</td>
<td>8.07 ±0.96</td>
<td>8.20 ±1.05</td>
</tr>
<tr>
<td>FEV1(liters)</td>
<td>3.41 ±0.37</td>
<td>3.37 ±0.55</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75%&lt;/sub&gt; (lit/sec)</td>
<td>3.88 ±0.87</td>
<td>4.36 ±1.19</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;75-85%&lt;/sub&gt; (lit/sec)</td>
<td>1.30 ±0.51</td>
<td>1.58 ±0.86</td>
</tr>
</tbody>
</table>

Discussion

When using measurement of carbon monoxide concentration in expired air for detecting recent smoking 8ppm can be kept as line dividing smokers and non smokers as suggested by Jarvis M et al and Im BG KS et al. Induction of a stress response protein, heme oxygenase-1 (HO-1) is one of the mechanisms protecting against an oxidative stress. Enhanced HO-1 protein expression may be due to the induction of enzyme by inflammatory cytokines and oxidants such as interleukins, tumour necrosis factor-α (TNF-α), interferon- γ, and H2O2 which are capable of inducing HO-1 expression in cell line and tissues. Induced HO-1 catalyzes the degradation of heme into bilirubin that can scavenge HO. in vitro as efficiently as α-tocopherol and the by-products of HO-1 activity are free iron and CO. Yamada N et al and Yasuda H et al have also reported that hemeoxygenase is present in the pulmonary vascular endothelium and alveolar macrophages. Up regulation of heme oxygenase-1 (HO-1) by oxidative stress and inflammatory cytokines in airways and lung inflammation has been reported, the cause of the increased levels of exhaled CO in patients with inflammatory lung diseases. These findings entail a role of endogenous CO in airway inflammatory diseases.
CO is a non-specific biomarker of tobacco exposure. Its half-life is short (2-6 hours).

Therefore if smokers are made to abstain from smoking for 8 hrs then the exhaled CO will be due to lung oxidative stress only. Therefore, measurement of exhaled CO is a simple method for detecting and monitoring cytokine mediated inflammation and oxidative stress in the respiratory tract. In our study we found out negative correlation between exhaled CO concentration and FEV1 % change after exercise challenge when values of all the subjects in the study were compared irrespective of their smoking status. Fig 6 demonstrates the correlation between exhaled CO and FEV1 % change after exercise challenge in the participants (r=-0.45, p=0.001). The exhaled CO concentration was greater in the smokers as compared to the healthy subjects (3.63±2.50 Vs 0.92±0.78). Negative co-relation between CO and FEV1 percentage change is suggestive of alteration of airway mechanics due to some underlying inflammation with the increment of concentration of CO in exhaled air. Relative increase in CO was reported in current smokers with COPD compared to healthy smokers matched for age and smoking habits by Pearce MS et al in 2005. This may indicate higher oxidative stress in the former group. Up regulation of heme oxygenase-1 (HO-1) by oxidative stress, inflammatory cytokines in airways and lung inflammation has been reported as the causes of the increased levels of exhaled CO in patients with inflammatory lung diseases.

**Conclusion**

A negative correlation was found between CO concentration in exhaled breath and improvement in FEV1 after exercise challenge when data of all the participants in the group were compared. Thus this negative correlation suggests that even in early asymptomatic smokers the decrease in improvement in FEV1 after exercise is due to oxidative stress due to smoking. Since, in our study all the smokers were smoking for not more than 5 years and none had ND score (Nicotine Dependence) more than 5 and all had a normal spirometry at rest, we can say that inflammatory changes start even before they are reflected in spirometry at rest. Breath CO analyser can be used to measure endogenous CO in so called “healthy smokers” as a tool to detect early inflammation and also to motivate them to quit smoking before the disease becomes irreversible.

**Ethical Clearance**- Taken from institutional Ethics committee for human research(IECHR), Medical college & SSG Hospital, Baroda.

**Source of Funding**- Self

**Conflict of Interest** - Nil

**References**