

# Hydrogen Peroxide in Exhaled Breath Condensate in Common Colds

Amey Paranjape<sup>1</sup>, Jairaj P. Nair<sup>2</sup>, Amita U. Athavale<sup>3</sup>, Shishir Singh<sup>4</sup>

<sup>1</sup>Corresponding Author, Dept of Chest Medicine and EPRC, Seth G.S. Medical College & KEM Hospital, Mumbai, India

<sup>2</sup>Department of Chest Medicine and EPRC, Seth G.S. Medical College & KEM Hospital, Mumbai, India

<sup>3</sup>Department of Chest Medicine and EPRC, Seth G.S. Medical College & KEM Hospital, Mumbai, India

<sup>4</sup>Department of Chest Medicine and EPRC, Seth G.S. Medical College & KEM Hospital, Mumbai, India

**Abstract:** Collection of exhaled breath condensate (EBC) is a non-invasive method for obtaining samples from lung for assessing biochemical lung function. EBC contains large numbers of mediators like Hydrogen peroxide ( $H_2O_2$ ). Concentration of these mediators is influenced by various respiratory diseases. The project was undertaken to determine the normal levels of the mediator  $H_2O_2$  in normal healthy subjects in an Indian population and in subjects with symptoms of common cold. It was established that common cold causes an increase in the level of  $H_2O_2$  and hence it has a confounding effect on the values of  $H_2O_2$  in EBC.

**Keywords:** Exhaled Breath Condensate (EBC), Common Cold, Hydrogen peroxide ( $H_2O_2$ )

## 1. Introduction

Exhaled breath condensate (EBC) is a non-invasive method for obtaining samples from lung for assessing biochemical lung function. EBC contains large numbers of mediators like Hydrogen peroxide ( $H_2O_2$ ), adenosine, ammonia, isoprostanes, leukotrienes, NO, peptides, cytokines and various anions.  $H_2O_2$  is produced after converting superoxide anions  $O_2^-$  to  $H_2O_2$  by superoxide dismutase in several cell types.<sup>[1,2]</sup> Hydrogen peroxide is released from both inflammatory and structural cells including neutrophils, eosinophils, macrophages and epithelial cells. Consequently,  $H_2O_2$  is a sensitive marker of oxidative stress in lung.<sup>[3]</sup>

Recently there has been increasing interest in investigations of lungs by non-invasive methods like bio-markers in exhaled breath condensate. EBC can also be collected in patients with poor ventilator function and for those on ventilator also. It seems essential to establish normal standards for Indian population as there is no data from this part of the world in regards to these breath condensate marker levels.

Common cold is a viral infection of upper respiratory tract. It is unknown whether viral colds contribute to exhaled hydrogen peroxide. This study will assess exhaled  $H_2O_2$  during common cold to establish the variations in  $H_2O_2$  levels in EBC in subjects with symptoms of common cold.

## 2. Methodology

This is a prospective case-control study involving 20 normal healthy subjects and 20 subjects diagnosed with common cold; both groups included subjects of either sex and were in the age range of 18-30 years. The study was performed at a tertiary care centre. The study commenced after obtaining written and informed consent from the subjects. Subjects with history of respiratory illnesses like bronchial asthma, tuberculosis, pneumonia were excluded.

The study was carried out over a period of 2 months. ECO Screen (Fig.1) was the basic device used for collection of exhaled air samples.



Figure 1: The Breath Condensate Collector (ECO Screen)

Stable cooling at approximately  $-10^{\circ}C$  assures quality of specimen even during longer collection times. ECO Vent (Fig.2) is an add on unit for ECO Screen and is recommended for standardizing exhaled breath measurements.



Figure 2: ECO Vent

ECO vent monitors breathing time, breathing volumes and breathing frequency. The ECO Check (Fig.3) measures the concentration of H<sub>2</sub>O<sub>2</sub> in exhaled breath condensate immediately after collection.

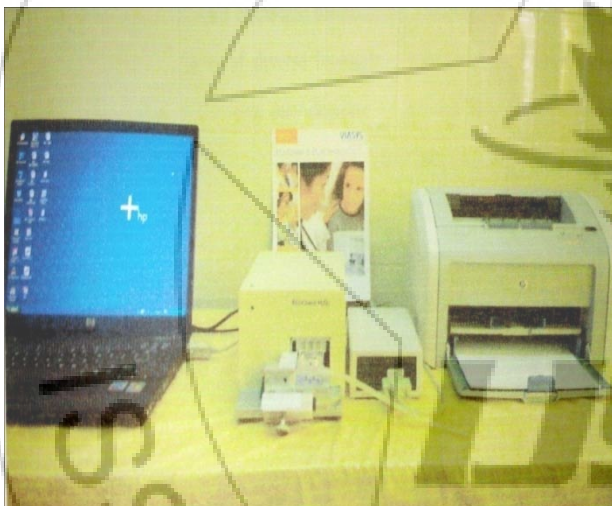


Figure 3: ECO Check

The ECO Check has a high-sensitive biosensor for the measurement of H<sub>2</sub>O<sub>2</sub> in concentrations of 15 to 10,000 nmol. EBC sample was done during tidal breathing using a nose-clip and a saliva trap defining cooling temperature and collection time (10 minutes is generally sufficient for obtaining 1-2 ml sample and is well tolerated by subjects) After collection, the sample was analyzed immediately for hydrogen peroxide levels.

The analysis was done with the ECO check. The biosensor uses the enzyme peroxidase to convert hydrogen peroxide. Hydrogen peroxide levels are obtained in few minutes and data was analyzed. Statistical analysis was done for the levels of hydrogen peroxide in EBC in normal healthy subjects (controls) and in subjects with symptoms of common cold for any variation in hydrogen peroxide levels in common cold. The unpaired 't' test was used to verify if the levels of hydrogen peroxide in the two groups is significant or not.

### 3.Results

Table 1: Hydrogen peroxide in exhaled breath condensate of normal healthy subjects (Table 1)

Sr. No	Age (yrs)	Sex	Vol. Exhaled (lit)	Time (min)	PEF (lit/s)	EBC H <sub>2</sub> O <sub>2</sub> levels (nmol/lit)
1	20	M	106.7	10.07	0.40	260
2	20	M	106.0	10.04	0.60	220
3	20	M	108.2	10.05	0.40	180
4	21	M	100.7	11.36	0.40	260
5	19	M	100.6	11.1	0.40	160
6	20	M	100.5	10.5	0.40	160
7	20	M	126.7	10.05	0.70	240
8	20	M	130.3	10.04	0.50	200
9	20	M	101.6	10.22	0.30	220
10	19	M	101.5	11.02	0.30	120
11	20	M	106.2	10.11	0.70	160
12	20	M	100.7	10.28	0.40	240
13	22	F	100.6	10.18	0.40	60
14	20	F	159.6	10.02	0.40	140
15	20	F	100.4	11.38	0.50	400
16	22	F	101	10.11	0.30	240
17	24	F	100.4	13	0.30	120
18	23	F	100.4	14.45	0.30	160
19	22	F	102.1	10.17	0.30	180
20	22	F	126.2	10.16	0.30	100

Table 2: Hydrogen peroxide in exhaled breath condensate of subjects with symptoms of common cold

Sr. No	Age (yrs)	Sex	Vol. Exhaled (lit)	Time (min)	PEF (lit/s)	EBC H <sub>2</sub> O <sub>2</sub> levels(nmol/l)
1	19	M	100.5	10.08	0.50	180
2	22	M	113.9	10.07	0.30	260
3	20	M	100.4	13.35	0.40	360
4	20	M	105.4	10.01	0.60	280
5	20	M	100.7	11	0.30	220
6	21	M	122.0	10.05	0.70	380
7	20	M	124.5	10.04	0.60	320
8	20	M	132.3	10.05	0.80	180
9	20	M	111.9	10.03	0.50	440
10	21	M	100.5	13.35	0.20	560
11	21	M	127.5	10.05	0.50	440
12	21	M	100.5	11.37	0.30	340
13	20	F	100.6	10.28	0.30	400
14	19	F	100.8	11.29	0.40	580
15	23	F	100.6	13.26	0.40	200
16	23	F	101.1	14.11	0.20	340
17	20	F	100.1	10.13	0.40	140
18	20	F	101	11.34	0.40	160
19	21	F	128.1	10.12	0.40	160
20	19	M	129.8	10.02	0.70	100

The mean value of hydrogen peroxide in EBC in normal healthy subjects was 191 nmol/lit, the range being 60-400 nmol/lit. The mean value of hydrogen peroxide in EBC in subjects with common cold was 302 nmol/lit, the range being 100-580 nmol/lit.

Unpaired 't' test was used to find whether the difference in between the mean values of H<sub>2</sub>O<sub>2</sub> levels in normal healthy subjects and in subjects with symptoms of common cold is significant or not.

The 't' value was found to be 3.18, therefore probability,

$P < 0.01$ . This suggests that hydrogen peroxide levels in all subjects with common cold were much higher than normal subjects (statistically significant).

#### 4. Discussion

Exhaled breath as a simple, non-invasive means to sample lower respiratory tract. The lining fluid of the lower respiratory tract contains various non-volatile and over 200 volatile substances.<sup>[5,6]</sup>

Exhaled breath analysis can be used to quantify inflammation and oxidative stress in the respiratory tract, in differential diagnosis of airway disease and in monitoring of therapy.<sup>[7]</sup> Hydrogen peroxide acts as a chemical signal molecule and as a marker of oxidative stress.<sup>[8]</sup> Hydrogen peroxide in exhaled breath condensate is elevated in respiratory diseases like bronchial asthma,<sup>[9]</sup> healthy smokers,<sup>[10]</sup> COPD,<sup>[11]</sup> bronchiectasis,<sup>[12]</sup> cystic fibrosis<sup>[13]</sup> acute respiratory distress syndrome<sup>[14]</sup> and pulmonary arterial hypertension.<sup>[15]</sup> The study by Jobsis R.Q.; Schellekens S.L. and co-workers concluded that  $H_2O_2$  in exhaled breath condensate is elevated during common cold and returns to normal within 2 weeks of recovery in healthy subjects. Hence, symptomatic upper respiratory tract inflammation may act as a confounding factor in studies of  $H_2O_2$  as a marker of lower airway inflammation.<sup>[16]</sup>

Present study showed that the difference between the mean values of two selected groups (healthy subjects and subjects with common cold) is statistically significant. Thus it is established that common cold has a confounding effect on the values of  $H_2O_2$  in EBC. Since the sample size used in this study is small, above results need to be confirmed by using a larger sample size.

#### References

- [1] Culpitt SV, Russel REK. The measurement of hydrogen peroxide in airway disease.
- [2] Eur Respir Rev 1999;68:246-48.
- [3] Conner GE, Salathe M, Foreteza R. Lactoperoxidase and hydrogen peroxide metabolism in the airway. Am J Respir Crit Care Med 2002;166:S57-S61.
- [4] Horvath I, MacNee W, Kelly FJ, et al. Haemoxygenase-1 induction and exhaled markers of oxidative stress in lung diseases. Summary of the ERS Research Seminar in Budapest, Hungary, Sept 1999. Eur Respir J 2001;18:420-30.
- [5] Horvath, J.Hunt, P.J. Barnes on behalf of the ATS/ERS Task Force on Exhaled Breath Condensate. Eur Respir J 2005;26:523-48.
- [6] Scheideler L, Manke HG, Schwulera U, et al. Detection of non-volatile macromolecules in breath: a possible diagnostic tool? Am Rev Respir Dis 1993;148:778-84
- [7] Manolis A. The diagnostic potentials of breath analysis. Clin Chem 1983;29:5-15
- [8] Sergei A, Kharitonov & Peter J. Barnes; Publisher-Taylor & Francis; Issue-Vol. 7, Number 1, January 1 2002; Pages: 1-32.

- [9] B. Chance et al. Hydrogen peroxide metabolism in mammalian organism- Physiol Rev 59(1979) 527-605.
- [10] Horvath I, Donnelly LE, Kiss A, et al. Combined use of exhaled hydrogen peroxide and nitric oxide in monitoring asthma. Am J Respir Crit Care Med 1998;158:1042-46
- [11] Guatura SB, Martinez JA, Santos Bueno PC, Santos ML. Increased exhalation of hydrogen peroxide in healthy subjects following cigarette consumption. Sao Paulo Med J 2000;118:93-98
- [12] Van Beurden WCJ, Dekhuijzen PNR, Harff GA, Smeenk FWJM. Variability of exhaled hydrogen peroxide in stable COPD and matched healthy controls. Respiration 2002;69:211-16.
- [13] Loukides S, Horvath I, Wodehouse T, Cole PJ, Barnes PJ. Elevated levels of expired breath hydrogen peroxide in bronchiectasis Am J Respir Crit Care Med 1998;158:991-94.
- [14] Worlitzsch D, Herberth G, Ulrich M et al. Catalase, myeloperoxidase and hydrogen peroxide in cystic fibrosis. Eur Respir J 1998;11:377-83.
- [15] Kietzmann D, Kah R, Muller M, Burchardi H et al. Hydrogen peroxide in expired breath condensate of patients with acute respiratory failure and with ARDS. Intensive Care Med 1993;19:78-81.
- [16] Mansoor JK, Schelegle ES, Davis CE et al. Analysis of volatile compounds in exhaled breath condensate in patients with severe pulmonary arterial hypertension. PLoS One. 2014 Apr 18;9(4):e95331
- [17] Jobsis R.Q., Schellekens S.L., Fakkkel-Kroesbergen A, et al. Source-Mediators of inflammation, Volume 10, Number 6, 1 Nov. 2001, pp.351-54.

#### Author Profile

**Dr. Amey Paranjape-** M.D. Pulmonary Medicine currently working as Specialty Medical Officer in Dept. of Chest Medicine & EPRC in Seth G.S. Medical College & K.E.M. Hospital.

**Dr. Jairaj Nair-** M.D. Pulmonary Medicine currently Assistant Professor in Dept. of Chest Medicine & EPRC in Seth G.S. Medical College & K.E.M. Hospital.

**Dr. Amita Athavale-** M.D. Pulmonary Medicine currently, Professor and Head of Dept. in Dept. of Chest Medicine & EPRC in Seth G.S. Medical College & K.E.M. Hospital.

**Dr. Shishir Singh-** M.B.B.S. Seth G.S. Medical College & K.E.M. Hospital.