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Hydrogen Peroxide in Exhaled Breath Condensate in Common Colds

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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.[1,2] 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.[3]

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 -10^0 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.

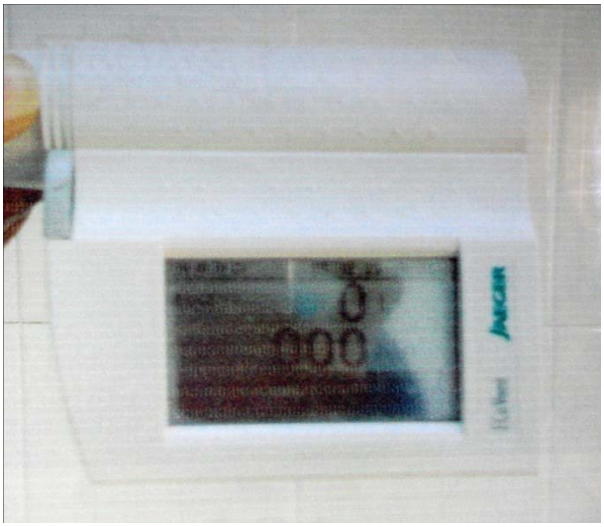

Figure 2: ECO Vent

ECO vent monitors breathing time, breathing volumes and breathing frequency. The ECO Check (Fig.3) measures the concentration of H_2O_2 in exhaled breath condensate immediately after collection.

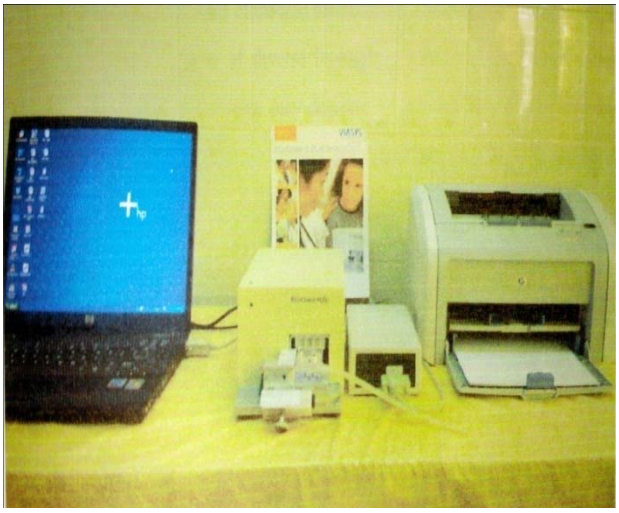

Figure 3: ECO Check

The ECO Check has a high-sensitive biosensor for the measurement of H_2O_2 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_2O_2 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_2O_2 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_2O_2 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.[5,6]

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.[7] Hydrogen peroxide acts as a chemical signal molecule and as a marker of oxidative stress.[8] Hydrogen peroxide in exhaled breath condensate is elevated in respiratory diseases like bronchial asthma,[9] healthy smokers,[10] COPD,[11] bronchiectasis,[12] cystic fibrosis[13] acute respiratory distress syndrome[14] and pulmonary arterial hypertension.[15] The study by Jobsis R.Q.; Schellekens S.L. and co-workers concluded that H_2O_2 in exhale 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.[16]

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.

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