

Original Article**Exhaled ethane concentration in patients with cancer of the upper gastrointestinal tract – a proof of concept study**Jo Etienne Abela^{1,2,*}, Kenneth D. Skeldon³, Robert C. Stuart¹, Miles J. Padgett⁴¹ Department of Surgery, Glasgow Royal Infirmary, Glasgow, UK;² Department of Surgery, University of Malta, Msida, Malta;³ Public Engagement with Science Unit, Office of External Affairs, University of Aberdeen, UK, and formerly of Department of Physics and Astronomy, University of Glasgow, Glasgow, UK;⁴ Department of Physics and Astronomy, University of Glasgow, Glasgow, UK.**Summary**

There has been growing interest in the measurement of breath ethane as an optimal non-invasive marker of oxidative stress. High concentrations of various breath alkanes including ethane have been reported in a number of malignancies. Our aim was to investigate the use of novel laser spectroscopy for rapid reporting of exhaled ethane and to determine whether breath ethane concentration is related to a diagnosis of upper gastrointestinal malignancy. Two groups of patients were recruited. Group A ($n = 20$) had a histo-pathological diagnosis of either esophageal or gastric malignancy. Group B ($n = 10$) was made up of healthy controls. Breath samples were collected from these subjects and the ethane concentration in these samples was subsequently measured to an accuracy of 0.2 parts per billion, ppb. Group A patients had a corrected exhaled breath ethane concentration of 2.3 ± 0.8 (mean \pm SEM) ppb. Group B patients registered a mean of 3.1 ± 0.5 ppb. There was no statistically significant difference between the two groups ($p = 0.39$). In conclusion, concentrations of ethane in collected breath samples were not significantly elevated in upper gastrointestinal malignancy. The laser spectroscopy system provided a reliable and rapid turnaround for breath sample analysis.

Keywords: Ethane gas, oxidative stress, laser spectroscopy, esophageal cancer, gastric cancer

1. Introduction

Oxidative stress is a state in which the rate of formation of highly reactive free radicals exceeds the rate of removal resulting in a wide range of complex redox reactions that mediate biomolecular injury (1). Free radicals cause degradation of polyunsaturated fatty acids (PUFAs) by a well-researched process known as lipid peroxidation (2). The by-products of this intricate chain reaction include aldehydes (e.g. malondialdehyde), conjugated dienes (e.g. isoprene) and alkanes such as ethane and pentane (3-6). These products have been

used to gauge the progress of lipid peroxidation *in vitro* and *in vivo*.

There is evidence showing high activity of several anti-oxidant enzymes and deficiency of anti-oxidant vitamins in a variety of cancers (7-11). In addition, several studies have explored the significance of lipid peroxidation products in a number of malignancies. High malondialdehyde levels have been found in esophageal, gastric and colorectal tumor tissue (7), as well as in the blood of patients suffering from breast (9) and gastric (10,12) cancer. On the other hand, breath testing studies have reported abnormally high concentrations of volatile organic compounds (VOC's) in lung (12-14) and breast cancer patients (15,16).

Backed by this evidence we set out to determine whether the exhalation of ethane (as a marker of oxidative stress *via* lipid peroxidation) is associated with upper gastrointestinal malignancy. Esophageal

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and gastric cancers have a notoriously bad prognosis when discovered late. At present, a curative strategy depends on diagnosis of early lesions that are amenable to radical surgical treatment. Therefore, this provides the rationale for research into the field of diagnostics in order to identify screening tests that can reliably detect early cancer.

2. Materials and Methods

2.1. Subjects

The Research and Ethics Committee of the North Glasgow University Hospitals NHS Trust gave ethical approval for this study. Twenty subjects with endoscopic and histo-pathologic evidence of upper gastrointestinal malignancy were recruited and constituted Group A in this study. Seven of these patients had adenocarcinoma of the esophagus and another 6 had squamous carcinoma of the esophagus. These patients had AJCC Stage II to III tumors. The remaining 7 patients had UICC Stage III to IV gastric adenocarcinomas. A further 10 subjects of similar age were recruited to serve as healthy controls, making up Group B. These subjects were selected from a University of Glasgow database for ethane levels in healthy adults. These controls had no history of gastrointestinal tract disease.

For the purpose of our study, the following were used as exclusion criteria in subject selection: i) anti-oxidant (including vitamin C, E and selenium supplements) medication; ii) steroid medication; iii) severe cardio-pulmonary disease precluding the collection of an adequate breath sample.

2.2. Samples

Three-liter Tedlar[®] bags with on-off valves were used for breath sample collection. These bags were flushed with hydrocarbon-free air (HCFA) (ethane < 100 parts-per-trillion) before being evacuated for breath sampling. In Group A, the samples were collected prior to surgical resection or endoscopic ablation of the tumors. In both groups subjects were asked to exhale into these bags in order to fill them up after having spent at least 30 min in their respective ward or clinic. We considered this to be the minimum time interval required for subjects to equilibrate with the ambient air (derived from our own unpublished data on healthy volunteers). Since cigarette smoke has been shown to significantly affect breath alkane measurements, samples from smokers were obtained at least 30 min after smoking to allow attainment of equilibrium, although it is accepted that this timescale may be too short in order to completely eliminate noise due to the smoke effect. In addition, one-liter Tedlar[®] bags were used to sample the ambient air for each subject recruited. Samples were processed typically one day

after collection in order to minimize errors caused by gas diffusion through the sample bags (1).

2.3. Measurement of ethane concentration

The average ethane concentration in the breath samples [e_{sam}] and the average ethane concentration b in the ambient air were measured using an ultra-sensitive tunable diode laser spectrometer (TDLS). This device (developed at the Department of Physics and Astronomy, University of Glasgow) comprises a cryogenically cooled lead-salt laser diode probing a target gas sample flowing through a multi-pass optical cell. The instrument is highly specific to ethane gas and reports ethane concentration with accuracy typically < 0.2 ppb and essentially in real time. The molecular vibrations excited in ethane by the laser are known absolutely and subsequent calibration is very reliable. The system is controlled by an integrated laptop computer and custom-designed software. Each sample could be measured in around 8 sec and an example trace for a breath sample opened up to the instrument is shown in Figure 1. In contrast, gas chromatographs or gas chromatography and mass spectrometry techniques require longer measurement periods and sample concentration for the ethane levels involved here. Ethane concentration is expressed here in parts per billion (ppb). The corrected average exhaled ethane concentration [e_{cor}] in the exhaled breath of our study subjects was calculated using the simple formula: $[e_{\text{cor}}] = [e_{\text{sam}}] - [b]$.

2.4. Statistics

An SPSS version 9.0 statistical software package was

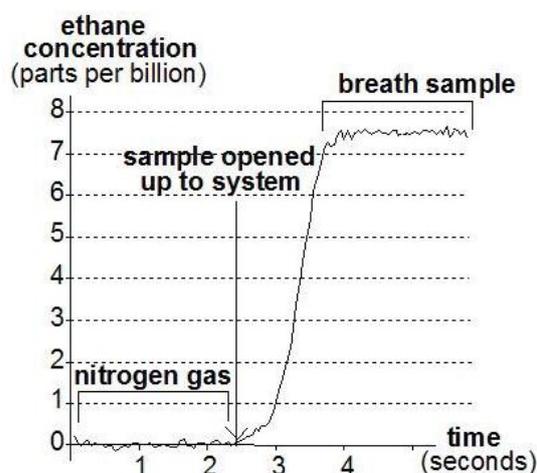


Figure 1. A typical trace of ethane gas in a breath sample from a patient in the study as measured by the TDLS. Note that this is the raw sample measured by the instrument and ambient background is not yet subtracted. The graph shows how rapidly the system registers the ethane in the sample bag. The deviation in the measurement at the plateau constitutes the intrinsic accuracy of the technique (around 0.2 ppb).

used to analyze our results. *P* values less than 0.05 were considered statistically significant.

3. Results

The observations and results for the two groups are summarized in Table 1. When compared for age, gender, a history of COPD and smoker status, there was no significant difference between the two groups (using Fisher's exact and Mann-Whitney tests as appropriate). As regards the corrected exhaled ethane concentration, there was no statistically significant difference between the two groups (Mann-Whitney test, $p = 0.39$). Likewise when the study population was divided into 4 subgroups according to cancer type (3 subgroups) and control status, there was no significant difference in ethane concentration (Kruskal-Wallis test, $p = 0.48$).

Figure 2 is a plot of $[e_{cor}]$ and age in the entire study population. The plot shows that there is no appreciable difference between patients and controls. It appears that with increasing age there is a downward trend in exhaled ethane concentration, however this observation did not reach statistical significance (Spearman's rank test, $p = 0.31$). We found no significant difference in ethane concentration between subjects with or without a history of COPD. Likewise there was no difference between smokers and non-smokers. The only significant difference was recorded when time from the last cigarette smoked was taken into account. Patients who had smoked 30-60 min before breath sampling were found to have a higher ethane concentration than non-smokers and/or those smokers who had abstained for more than 1 h before sampling (Kruskal-Wallis test, $p = 0.03$). Exclusion of the former group of smokers from our analysis of the difference in $[e_{cor}]$ between Groups A and B, was still not conducive to statistical significance (Mann-Whitney test, $p = 0.26$).

4. Discussion

Ethane (C_2H_6) is considered to be one of the best markers of lipid peroxidation (2). The main endogenous source of ethane in the body is peroxidation

of n-3 PUFAs, specifically the cleavage of the 16-hydroperoxide of 9,12,15-linoleic acid. Conversely, pentane (C_5H_{12}) is evolved from degradation of the more abundant n-6 PUFA's. Both gases are saturated, aliphatic hydrocarbons, which are sparingly water-soluble. Ethane has a lower molecular weight that renders it poorly lipophilic as well. This also renders ethane more inert than pentane; it is less tissue-soluble and less likely to be metabolized. Ethane levels, therefore, exhibit less day-to-day variation than pentane. Preliminary studies have shown typical day to day variation of exhaled ethane in healthy adults in this age group to be in the range of 0.5 ppb compared to a mean of around 3 ppb (19). For this reason ethane was our preferred marker for oxidative stress.

Ethane is present in the air at around 1.5 ppb concentration in the Northern Hemisphere. For any exhaled breath sample, a fraction of the ethane level will be due to this environmental component. Since our subjects were submitting expiratory volumes from total lung capacity (TLC) to residual volume (RV), the dead space volume introduced an exogenous ambient ethane component causing a systematic error. Contrary to other studies employing HCFA breathing techniques or dead-space eliminating sampling methods, we opted for an ambient air breathing method and a simple bag sampling technique (20). In order to correct the error introduced by the inhaled ethane fraction, we firstly allowed the subject to come into equilibrium with the ambient air before breath sampling. Subsequently, we subtracted the ambient ethane concentration from the exhaled breath sample concentration. We consider our strategy to be simple, straightforward and adequate for the purpose of a proof of concept investigation. In this study we accepted breath samples only where the accompanying ambient level was less than 5 ppb in order to keep a significant proportion of breath ethane over ambient level and reduce errors.

Table 1. Summary of the results

	Group A (cancer)	Group B (control)
Number of subjects	20	10
Mean age, yrs ($p = 0.29$)	72 ± 2	67 ± 4
Male : Female ratio	2.3:1	2.0:1
Smokers ($p = 0.68$)	30%	20%
COPD ($p = 0.63$)	30%	10%
Mean of corrected average exhaled ethane concentration $[e_{cor}]$, ppb ($p = 0.39$)	2.3 ± 0.8	3.1 ± 0.5

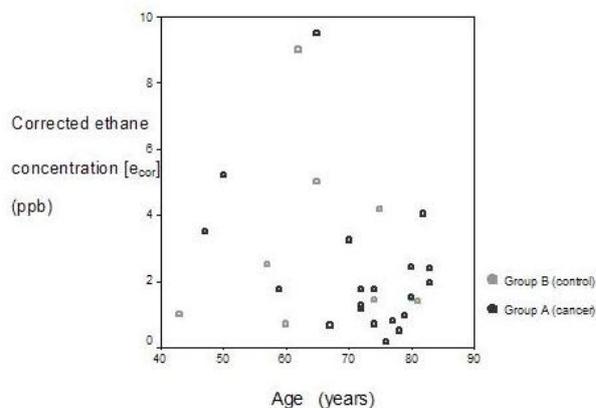


Figure 2. Ethane concentration $[e_{cor}]$ and age. There is no appreciable difference in $[e_{cor}]$ between patients and controls. There appears to be a downward trend with age but this was not statistically significant ($p = 0.31$).

Abnormally high breath ethane levels have been reported in several disorders including smoking (21,22), asthma (23), chronic obstructive pulmonary disease (COPD) (24,25), cystic fibrosis (26), myocardial infarction (2), inflammatory bowel disease (27,28), HIV infection (29). Interestingly, elevated concentrations have also been noted in less well-understood conditions such as multiple sclerosis (30), schizophrenia (31), and childhood attention deficit (32). To date ethane has not been employed in cancer studies. High concentrations of o-toluidine were detected in association with several tumours (33). Pentane was found to be abnormally high in a breast cancer (15). Another breast cancer study used a set of VOC's and reported that a three-dimensional display of the alveolar gradients of these gases had a better negative predictive value than actual mammography (16). In one lung cancer study, butane was identified as the best single discriminator for lung cancer (12).

Our results failed to show a useful association between exhaled ethane concentration and invasive upper gastrointestinal cancer. This result is significant because there is ample evidence that these conditions are indeed linked with the state of oxidative stress. The only significant elevations we recorded occurred soon after smoking and this observation has already been reported (21). This phenomenon is probably due to damage of the respiratory mucosa mediated by free radicals in cigarette smoke (22). We also note that the mean ethane concentration in our control group (3.1 ± 0.5 ppb) was higher than was recently reported by Paredi and colleagues (0.88 ± 0.09 ppb) (25,26). However, the control population in these studies was significantly younger than our own (mean age 33 and 74 years, respectively).

5. Conclusion

This is the first study employing a tunable laser-diode spectrometer to explore the relationship between upper gastrointestinal malignancy and ethane concentration in exhaled breath. We conclude that analysis of breath ethane based on a simple bag collection procedure and after taking account of ambient conditions, does not provide a useful discriminator for invasive esophageal and gastric cancer.

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