^{1}H MRS of Sputum for the Non-invasive Diagnosis of Lung Cancer

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INTRODUCTION: Lung cancer is the leading cause of cancer death among both men and women in North America. The 5-year survival rate for non-small cell lung cancer (NSCLC) is 15% and that for small cell lung cancer (SCLC) is only 6%. One of the reasons for the low survival rate of lung cancer patients is that the patients are asymptomatic in the early stage of the disease, and by the time they develop symptoms such as prolonged coughing, chest pain and/or coughing up blood, the disease will be in the advanced incurable stage. Presently, there are no effective screening tests for the early diagnosis of the disease. Currently available diagnostic techniques include chest X-ray, CT, magnetic resonance imaging (MRI), bronchoscopy, lung biopsy, and sputum cytology. In chest X-ray, the tumors must reach a size of 0.8-1.0 cm in diameter to be visible. Low dose CT scan is capable of detecting small nodules but is also associated with a high false-positive rate [1]. MRI is primarily used to determine the stage and spread of the cancer once it is diagnosed. To localize small and radiologically-occult early cancers, the only diagnostic tool currently available is bronchoscopy. White light bronchoscopy has a sensitivity of only 30% in the detection of cancer in the early stage, whereas fluorescence bronchoscopy yields higher sensitivity (~70%) [2]. However, the drugs used in fluorescence bronchoscopy have been shown to have serious side effects of dermatologic photosensitivity. Histopathologic analysis is considered to be the gold standard, but the procedures involved to obtain a biopsy are generally invasive and prone to sampling errors. Sputum cytology is currently the only non-invasive method that can detect pre-malignant lesions or carcinoma in situ in the tracheobronchial tree. However, it has a sensitivity of only 60% and a high false positive rate. Recently, there has been some work on identifying genetic markers for lung cancer in sputum samples, with the studies still underway [3]. In the present study, we have analyzed sputum samples from patients with NSCLC and SCLC, and compared them with normal controls, making use of ^{1}H MR spectroscopy.

MATERIALS AND METHODS: Sputum samples were collected from 15 subjects (NSCLC, n=6; SCLC, n=3; normal controls, n=6) at the Ottawa Hospital. Samples were frozen and shipped to the NRC Institute for Biodiagnostics in Winnipeg for MRS analysis. The sputum samples were thawed and treated with 2M NaCl to reduce their viscosity and facilitate the MRS analysis. All MRS experiments were performed on an Avance 360 MHz Spectrometer (Bruker Instruments) with no spinning. The following acquisition parameters were employed in all experiments: NS (number of scans) = 256; P1 (90° pulse) = 6 µsec, PL9 (presaturation power) = 40 dB, TD (number of points in time domain) = 32K, D1 (relaxation delay) = 5.0 sec, SW (spectral width) = 4990 Hz, and AQ (acquisition time) = 2.8 sec. A split aliquot portion of the sample was also used for cytology.

RESULTS & DISCUSSION: Figure 1 shows typical 1D ^{1}H MR spectra of sputum samples obtained from (a) a normal control and (b) a lung cancer patient. Some common biochemicals found in the sputum of both normal control and lung cancer patients have been labeled in Figure 1b. Comparing the spectral patterns of sputum samples from both groups, it is evident that the spectrum of the normal control subject shows the presence of glucose, whereas glucose was absent in that of the lung cancer patient. It is worth emphasizing that glucose was absent in <u>all</u> of the nine patients with cancer. We observed the presence of glucose in 4/6 normal control subjects. Cytologic analysis of the samples showed that only 10/15 samples were actually sputum, with the remaining 5 samples being saliva. This is due to the difficulty of getting samples from deep respiratory tracts. Of the two control samples that didn't show glucose, one was actually confirmed to be saliva on cytology. The other sample that came from a normal control but showed an abnormal spectrum was found to have atypical cells on cytology, which may indicate the presence of some premalignant changes. While cytology and the DNA-based methods look at the changes in the cells shed from the respiratory tract, our method has the potential to pick up any biochemical changes indicative of malignancy in any of the secretions – even in the absence of cells. Hence, we have included all the 15 samples in our analysis and based on the presence/absence of glucose, we obtained an overall accuracy of 87% (i.e., 13/15 agreement with clinical diagnosis). Excluding all the saliva samples (n=5) and focusing only on the sputum samples (n=10), we obtained an overall accuracy of 90% (i.e., 9/10 agreement with clinical diagnosis), with the only disagreement being the subject whose sputum sample showed atypia. It is interesting to note that the cytologic results on the sputum samples were in agreement with the clinical diagnosis only in 6 out of 10 cases.

The reason for the absence of glucose in the sputum samples obtained from lung cancer patients could be due to an increased rate of glycolysis in the cancer cells. There is an increased demand for glucose in cancer cells and hence the glucose transporters are over-expressed in human malignancies enhancing glucose influx into the cancer cells. The cancer cells rely entirely on glycolysis for their metabolic demands due to their inability to produce ATP from pyruvate since they have a defective Krebs cycle. The glycolytic ATP production requires availability of NAD$^+$, which is supplied in cancer cells by the oxidation of NADH during the reversible transformation of pyruvate to lactate [2]. This transformation is catalyzed by a key enzyme, lactate dehydrogenase-5 (LDH-5), which is generally over-expressed in lung cancer patients [2]. In the present study, we have observed lactate in both the normal control and cancer patients, and could not make a correlation between the absence of glucose and the levels of lactate in the sputum samples from cancer patients. It may be possible to obtain such a correlation if the study is performed on a larger patient cohort.

CONCLUSION: The absence of glucose in the sputum samples from lung cancer patients could be due to an increased rate of glycolysis, and the present observation may have diagnostic potential in the rapid and non-invasive diagnosis of lung cancer. Although these results are very promising, they are preliminary and need to be validated with a larger patient cohort.

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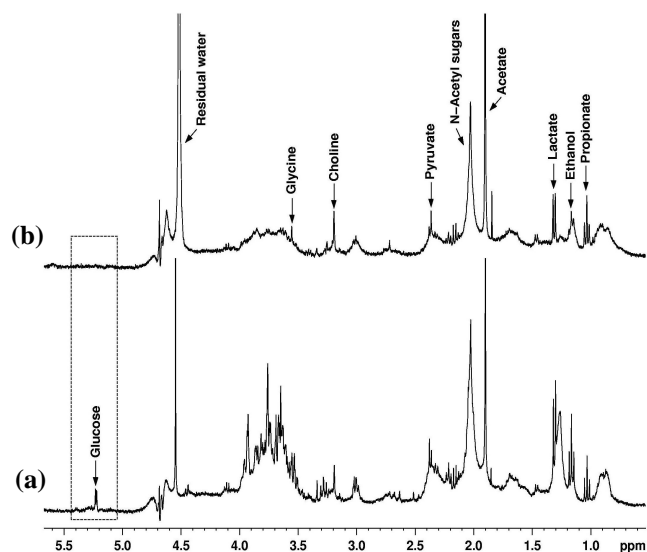4. Koukourakis MI, Giatromanolaki A, Sivridis E, et al. *Br J Cancer* 2003; **89**:877-885.

Figure 1: ^{1}H MR spectra of sputum samples from (a) Normal control and (b) Lung cancer patient (NSCLC) showing absence of glucose .