A PRELIMINARY STUDY ON A FLUORESCENCE SENSOR FOR EFFICACY ASSESSMENT OF CHEMOTHERAPY TREATMENT IN LUNG CANCER PATIENTS

K.B.G.S. Upendra[1], N. Jeyakumaran[2], C.D. Jayasinghe[3], M.D.P. De Costa[4], R. Senthilinithy [1]*

[1]Department of Chemistry, The Open University of Sri Lanka
[2]Apeksha Hospital,National Cancer Institute, Maharagama
[3]Department of Zoology, The Open University of Sri Lanka
[4]Department of Chemistry, University of Colombo

In Sri Lanka lung cancer patients generally present themselves at the late stages of the cancer and consequently they are treated with chemotherapy irrespective of the type of cancer. Both non-small cell lung cancer and small cell lung cancer are treated with different sets of chemotherapy and the response to the chemotherapy is monitored after the treatment cycle using the images. Hence, there is an urgent need for a biomarker to detect the efficiency of chemotherapy. DNA methylation-based biomarkers have proven to be more specific and sensitive than commonly used protein biomarkers. Hypermethylation of CpG islands is a sign of malignant growth and profiling of methylation in circulating tumour DNA (ctDNA) has gained increasing attention as potential biomarkers.

Accordingly, this study aims at developing a fluorescence biomarker that could potentially be used to detect the epigenetic alterations in circulating tumour DNA (ctDNA) isolated from blood plasma of lung cancer patients. The blood samples were collected at the pre and post chemotherapy period of 5 lung cancer patients admitted to the Apeksha Hospital. The ctDNA isolated from the patient blood plasma were subjected to the Fluorescence assays. The attempt made to develop a fluorescence probe to detect chemotherapy efficacy from ctDNA with 1,10-Phenanthroline-5-amine was very successful. The binding constant was determined by UV-Visible spectroscopic method.

The blood samples taken after chemotherapy treatment was treated with 1,10-Phenanthroline and 1,10-Phenanthroline-5-amine. The binding constant (K_b) of the resultant solution and number of binding sites (n) in ctDNA strands were determined using UV-Visible spectroscopic method and fluorescence spectroscopic method. It was found that 1,10-Phenanthroline-5-amine (K_b=49.3±0.05) has the greatest binding affinity in ctDNA than 1,10-Phenanthroline (K_b=22.6±0.05) which was determined by fluorescence spectroscopic method whereas the binding affinity of that 1,10-Phenanthroline-5-amine (K_b=45.3±0.05) and 1,10-Phemamthroline (K_b=19.6±0.05) by UV-Visible spectroscopic method. The higher number of binding sites in ctDNA 1,10-Phenanthroline-5-amine (n=10) than 1,10-Phenanthroline (n=1.2) were determined by fluorescence spectroscopic method. Also, 1,10-Phenanthroline-5-

amine has a higher Stern-Volmer constant (K_{SV}=13.7) compared to 1,10-Phenanthroline (K_{SV}=1.66).

The same procedures were carried out for the blood samples taken before chemotherapy treatment with 1,10-Phenanthroline and 1,10-Phenanthroline-5-amine. The ctDNAin pre chemotherapy samples with the above two compounds show less binding affinity and lower number of binding sites compared with the post chemotherapy sample. Thus it can be concluded that1,10-Phenanthroline-5-amine is a better fluorescence marker than 1,10-Phenanthroline to determine the efficacy of the chemotherapy and hence could be developed as a potential epigenetic marker. An extended study with a higher number of patients with shorter chemotherapy period involving a series of testing within the chemotherapy cycle will further validate the use of fluorescence biomarker for clinical applications.

Keywords: Circulating tumour DNA (ctDNA), Stern-Volmer constant, Fluorescence sensor, 1,10-Phenanthroline-5-amine.

**Corresponding author: rsent@ou.ac.lk*