ABSTRACT

Cancer is one of the major causes of illness and death worldwide. There are 7.6 million new cancer cases globally, of which 52% occur in developing countries. The magnitude of the problem of cancer in India in terms of sheer number is most alarming. The estimated new cases of cancer in India per year are nearly 6.5 lakhs. Cancer is due to proliferation of abnormal cells, which develop from body’s own cells. As they develop from body’s own cells, cancer cells are not recognized as foreign and continue to grow and invade without being attacked by body defenses. Cancers become dangerous if they are detected late, that is at a stage when they have spread to other organs or have caused destruction to surrounding tissues. The secondary growths interfere with the functioning of the particular organ or site involved. On the other hand, if the cancer is detected at an early stage, it can often be cured either by means of surgery or by other therapeutic means available. Hence early diagnosis of cancer is mandatory for effective and successful treatment of this disease.

In spite of the recent advances in diagnostic oncology, early diagnosis of cancer remains a challenge to the medical community. The conventional methods of cancer diagnosis such as X-rays, CT-scan etc. use ionizing radiation, which produces adverse effects in the normal tissues surrounding the tumor. Other diagnostic modalities such as magnetic resonance imaging (MRI) and positron emission tomography (PET) are expensive. Histopathology is still considered as the gold standard for the diagnosis of cancer. However, it is an invasive procedure and requires removal of tissue from the body. Also, it requires a multi-stage process, which is time consuming and requires personnel skill. In this regard, there exists a clear need
for a non invasive and real time diagnostic technique which could facilitate early diagnosis of cancer and overcome the demerits of the existing modalities of cancer diagnosis.

Under these circumstances, tissue optics and its applications have emerged as promising techniques, which can provide opportunities for diagnosis focusing at the manifestations of predisease within intact tissues. Over the past two decades, various optical spectroscopic techniques have been developed as effective tools for diagnosing human premalignant and malignant lesions. Among the various diagnostic techniques, fluorescence spectroscopy and its complementary techniques offer the benefits of less invasiveness, real-time diagnosis and easy operation. Fluorescence spectroscopy of tissues is based mainly on the fluorescence spectral characteristics of intrinsic fluorophores or labelled extrinsic fluorophores. Currently, photophysical properties of intrinsic biomolecules and their structure have been considered as useful parameters to study various alterations in the functional, morphological and microenvironmental changes in cells and tissues.

As many metabolic changes in the body will be indirectly reflected in blood, which has many intrinsic fluorophores, native fluorescence spectroscopy of blood and its constituents has emerged as one of the complementary techniques in analytical haematology. However, only limited studies have been reported on the applications of this technique to discriminate normal from pathological conditions. Although these reported studies support the hypothesis that native fluorescence spectroscopy may provide a promising method of measuring pathobiochemical changes in blood for disease diagnosis, research in the field of disease diagnosis by optical spectroscopy of blood is still in the primitive stage. Further, the reported works do not suggest a
comparison of the fluorescence emission characteristics of blood plasma / sera of malignant subjects with respect to nonmalignant diseases and healthy subjects. Moreover, to the best of our knowledge, no reported work is available on the comparison of different techniques such as fluorescence emission and excitation spectroscopy, synchronous luminescence spectroscopy and excitation and emission matrices of blood plasma of malignant and non malignant subjects with respect to normal. Besides, the study on optical spectroscopy of blood in diagnostic oncology has not yet been fully optimized both experimentally and statistically.

In this context, the present thesis is aimed at studying the native fluorescence characterization of blood plasma of healthy subjects, patients with cancer of different origins, such as oral cavity and upper gastrointestinal tract and non-malignant liver diseases. Characteristic spectral differences were observed between normal and diseased subjects in the fluorescence emission and excitation spectra measured at different wavelengths of excitation / emission. These spectral differences were attributed to possible changes in the fluorescence characteristics of different intrinsic fluorophores present in the blood plasma due to morphological and/or microenvironmental changes in diseased subjects with respect to normal.

Detailed statistical analysis of the spectral characteristics was also carried out using stepwise linear and multiple discriminant analysis. Better values of specificity and sensitivity were obtained in the discriminant analyses performed at different excitation and emission wavelengths. Normal and oral cancerous subjects were classified with an overall accuracy of 95% at 405 as well as 420 nm excitations. When compared to advanced oral cancerous subjects, early stages of oral cancer were classified with better accuracy thereby showing the potentiality
of this technique in the early diagnosis of oral cancer. Gastrointestinal cancerous subjects were
discriminated from normal with an overall accuracy of 90% at 420 nm excitation. When
compared to gastrointestinal cancerous subjects, oral cancerous subjects showed better
discrimination from normal. This may be due to the wide range of cancers included under the
group of gastrointestinal cancer in the present study. Liver diseased subjects were classified
with an overall accuracy of 100% at 405 as well as 420 nm excitations. Oral cancerous subjects
were discriminated from liver diseases and normal subjects with an overall accuracy of 92% at
420 nm excitation whereas gastrointestinal cancerous subjects were discriminated with an
overall accuracy of 87% for the combination of 400 and 405 nm excitations. A combined
discriminant analysis across the four groups, normal, oral cancer, gastrointestinal cancer and
liver diseases resulted in an overall accuracy of 82%. The results of the present study
demonstrate the potentiality of the present technique not only in discriminating cancerous
subjects from normal but also, cancerous subjects from non-cancerous subjects. It is suggested
that extensive research in this direction with large scale sample population is mandatory to
optimize this technique for clinical trial.

In order to find whether the blood plasma and tissues during regeneration exhibit
similar fluorescence spectral characteristics as cancerous subjects, a pilot study was carried out
on the fluorescence spectroscopic characterization of blood plasma and liver tissues of animals
subjected to partial hepatectomy, which is surgical removal of part of the liver to stimulate liver
regeneration. The blood plasma and liver tissues of regenerating animals showed characteristic
fluorescence in the red region as observed in the case of cancerous subjects and this red
emission may be attributed to endogenous porphyrins. However, the emission wavelength of
this characteristic red emission was different in regenerating animals (around 620 nm) when compared to cancerous subjects (around 630 nm). This difference in the wavelength of porphyrin emission in regenerating animals and cancerous subjects may be attributed either due to different types of porphyrins contributing to the emission characteristics or due to their altered microenvironment changes in these two groups. Hence, the observed results suggest the possibility of differentiating the spectral characteristics of blood plasma and tissues during liver regeneration from that of cancerous subjects. Also, this study suggests the possibility of using native fluorescence spectroscopy for possible assessment of liver regeneration, which is mandatory in the clinical situation as a prognostic tool.

Besides the applications of steady state fluorescence spectroscopy in the characterization of diseased and non diseased tissues, time-gated fluorescence spectroscopy also provides additional information in the qualitative analysis of spectral characteristics of tissues as it allows to depict individual components of complex fluorophores on the basis of their decay times. Surgical removal of brain tumor is the most common initial treatment received by brain tumor patients. Hence, the goal of brain tumor resection procedures is to maximize tumor removal with minimal neurological damage. To achieve this goal, accurate intraoperative identification of brain tumor margins during craniotomy is required. In this regard, a preliminary study was carried out on the comparison of steady state and time gated fluorescence spectral characteristics and steady state fluorescence images of normal and solid tumor tissues from human glioblastoma patients treated with 5-ALA. The time gated spectral measurements were compared with that of steady state measurements and it is found that time gated measurements provide additional information about the relative contribution of different
intrinsic fluorophores in normal and tumor tissues, when compared to steady state measurements. A comparison of the present study with the reported results of steady state spectroscopy of tissues showed contradictions in the relative concentration of the intrinsic fluorophores in normal and tumor tissues. This suggests the need for detailed studies in this direction.

Apart from fluorescence spectroscopy, steady state diffuse reflectance spectroscopy is found to be one of the simplest spectroscopic techniques for studying biological tissue. This technique is found to be a valuable supplement to standard histologic techniques, which require multiple-stage sample preparation process and hence time consuming. Several researchers have used diffuse reflectance spectroscopy to study biological tissues. However, to the best of our knowledge, no reported work is available on the in vivo diffuse reflectance characterization of oral tissues of high risk smokers with non smoking population as well as oral cancerous subjects and a comparison with the in vitro studies using a conventional spectrofluorometer. In this context, a pilot study was carried out in this direction to find the possibility of discriminating smokers at a high risk of developing precancerous conditions of oral cancer such as leukoplakia in a mass population, using in vivo diffuse reflectance characteristics of oral mucosa. The possibility of discriminating diffuse reflectance characteristics of oral cancer with respect to normal tissues was also estimated both in vitro and in vivo. The specificity and sensitivity of the present technique was evaluated using stepwise discriminant analysis. High risk smokers were discriminated from non smokers with an overall accuracy of 83%. Oral cancerous subjects were discriminated from normal with an overall accuracy of 94%. High risk smokers were discriminated from oral cancerous subjects with an overall accuracy of 96%.
Though the present study was carried out on less number of samples, the results of the present study suggest that diffuse reflectance spectroscopy shows promising possibilities in the discrimination of oral cancer as well as high risk smokers from normal subjects. However, detailed studies on large scale population is required to confirm the present observations and to optimize this technique for further possible applications in the clinical situation.