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Research Article

STUDY OF HYPOXIA IN TUMOUR USING UV-VISIBLE SPECTROSCOPY, ATOMIC EMISSION SPECTROSCOPY AND MAGNETIC RESONANCE IMAGING

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ABSTRACT

Hypoxia was identified as a micro environmental component of tumours over 60 years ago and was immediately recognized as a potential barrier to therapy through the reliance of radiotherapy on oxygen to elicit maximal cytotoxicity. Over the last two decades both clinical and experimental studies have markedly enhanced the understanding of how hypoxia influences cellular behaviour and therapy response. Furthermore, researchers have confirmed early assumptions that low oxygenation status in tumours is an exploitable target in cancer therapy. In this paper, we describe the methods of diagnosis and measuring tumour hypoxia using UV-Visible, Atomic emission spectroscopy and Magnetic resonance imaging. The study of oxyhaemoglobin content of healthy and cancerous blood was carried out using UV-Visible spectroscopic techniques. The estimation of the amount of iron content in cancerous blood patients was done by atomic emission spectroscopy. The results revealed a decrease in the trace elemental iron concentration. This in turn decreases the oxyhaemoglobin level in leukemic patients. The deficiency of trace elemental iron lead to hypoxia, and is further confirmed by Magnetic Resonance Imaging of kidney of the patients.

INTRODUCTION

Leukaemia is a disease of unknown aetiology characterized by an uncontrolled and abnormal proliferation of one or more of white blood cells and their precursors in the bone marrow associated with a marked increase in the white cells and their precursors in the peripheral blood. It could be said to be a neoplasia, a malignancy or 'cancer' of the white blood cells (1). It used, to be the part of the definition of the disease that it was a 'fatal' condition, that no cure was possible. As the tumour initially grows in size, it is dependent on the pre-existing blood vessels to supply oxygen, and hence there are regions which become hypoxic, i.e. lacking in oxygen. This hypoxia can be caused both by reduced perfusion, either general or local, resulting in heterogeneous blood flow across the tumour, and by increased inter capillary distances leading to larger average diffusion distances for oxygen. Cancer treatment may cause anaemia, which is a third cause of tumour hypoxia, related to the reduced oxygen-carrying ability of the blood (2). There are two types of hypoxia: chronic and acute. Acute hypoxia is a sudden reduction in available oxygen, and is usually caused by fluctuations in blood flow, whereas chronic hypoxia is more ongoing and in tumours is usually caused by insufficient vasculature.

The term hypoxia has been extensively used to describe a state of insufficient oxygen, which can be present in tumour as well as normal tissues and wound. Medical imaging tests are used to assess the condition of the internal organs. These tests may be performed as part of the diagnosis to rule out other conditions that may be causing symptoms, to look for possible signs of leukaemia in internal organ, or to prepare for treatment or as part of follow-up care. MRIs use strong magnetic fields and high-frequency radio waves to create three-dimensional images and two-dimensional cross sections of the internal organs. MRI may be performed on leukaemia patients to evaluate the condition of the internal organs and look for signs of leukaemia. In the 1950s, Gray showed that increased oxygen tension correlated with increased cell degeneration *in vitro* following exposure to X-rays, and that increasing levels of inspired oxygen for mice resulted in greater tumour regression (2). This matched previous findings in plant and insect tissues. The role of oxygen in varying radio sensitivity of cells has since been investigated in bacteria (3). It is thought that this relationship between hypoxia and lower levels of cell death following irradiation is primarily a result of a reduction in O_2-induced propagation of free radical damage, but evidence shows hypoxia-mediated proteomic and genomic changes are likely to also contribute towards tumoural radioresistance by

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increasing the levels of heat shock proteins (HSPs) (4). Heat shock proteins are responsible for maintaining cell function during stress by stabilising and re-folding denatured proteins, and generally act as molecular chaperones by aiding protein transport. Hypoxia has since been repeatedly shown to have a negative effect on outcome of radiotherapy in patients.

In the early 19th century August Krogh carried out the first studies into the diffusion of gases through animal tissue (5), and calculated the partial pressure of oxygen required to supply these tissues. In 1955 Gray and Thomlinson proposed the existence of hypoxic regions. Tissue is considered to be hypoxic when the partial pressure of oxygen is insufficient for cellular metabolic processes. Detrimental changes have been observed at oxygenation levels of 8-10 mmHg, and 10 mmHg is thought to be the threshold above which levels of ATP remain constant. Below around 0.5 mmHg, mitochondrial oxidative phosphorylation is limited. Usually in hypoxic regions there is a switch from oxidative phosphorylation to anaerobic glycolysis as the primary metabolic pathway, leading to production of pyruvate, an increase in glucose demand and a reduction in O_2 demand. In a hypoxic environment, pyruvate ferments to produce lactate, which is the dissolved form of lactic acid. This change in metabolism within tumour was first noted by Otto Warburg in the 1920s (6).In addition to causing a change in metabolism, hypoxia is known to result in a more aggressive tumour phenotype. Overall, hypoxia leads to a greater likelihood of local invasive growth, perifocal spread and regional and distant spread of tumourcells. Hypoxia has been found to predict for survival and local control independently of tumour stage and nodal status. As already discussed hypoxia can cause anemia in the cancer patients. Anaemia leads to death of blood cells and affects the kidney in cancer patients.So to confirm the above facts, this paper has been focused to (i) Diagnose the oxyhaemoglobin content of normal blood and cancerous blood by UV-Visible spectroscopic technique, (ii) Estimate the amount of iron content in cancerous blood patients by Atomic emission spectroscopy, (iii) Diagnose the kidney of cancer patients using Magnetic Resonance Imaging (MRI) for studying the level of hypoxia.

METHODS AND MATERIALS

Blood samples are collected from normal and leukaemic patients of same blood group. Plasma is obtained and is diluted with normal saline water and is fed into UV- 3600 double beam spectrophotometer. The spectra were scanned between 500 and 200 nm. The results obtained are interpreted with respect to the absorbance values of λ_{max} of the oxyhaemoglobin. The spectra are presented in Figs 1- 6 and the results obtained are given in Table 1.Same samples were used to estimate the micro elemental iron using atomic emission spectrometer. The concentration of iron was estimated in the plasma of the diseased subjects by 3410 ICP Atomic emission spectrometer. The results are tabulated in the table 2. Cancer patients were scanned using Philips Achieva system at1.5 tesla. All images were acquired using a 16 channel torso wrap-around coil during free breathing without breath holding. High resolution anatomical images were acquired. Various modalities of the kidney are shown in Figure 7.

Table1 UV Visible absorption characteristic of oxyhaemoglobin

S.No.	Samples	Wavelength Maxima of oxyhaemoglobin (nm)	Absorbance
1.	Normal	413.2	2.253
2.	Leukaemia 1	410	0.123
3.	Leukaemia 2	410	0.167
4.	Leukaemia 3	410	0.158
5.	Leukaemia 4	413	0.322
6.	Leukaemia 5	413	0.422

Table 2 Trace elemental concentration of acute leukaemia blood

S.No.	Acute Leukaemia blood µgms/ml
Leukaemia 1	1.301
Leukaemia 2	3.785
Leukaemia 3	2.832
Leukaemia 4	5.632
Leukaemia 5	4.578

Normal value of trace elemental Iron 10µgms/ml

RESULTS AND DISCUSSION

The characteristic peaks and their absorbance values gives information about the nature of the blood samples. The formed elements of healthy blood exhibit their wavelength maxima as given in the Fig 1and table 1.

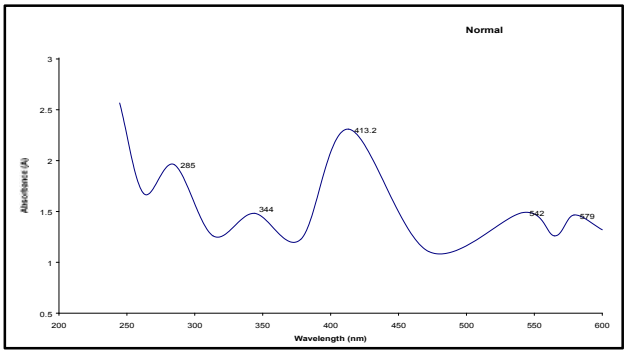

Fig 1 UV spectrum of normal blood

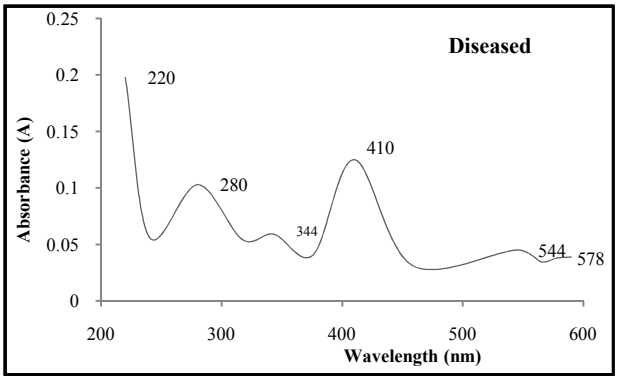

Fig 2 UV spectrum of leukaemia 1 blood

The wavelength region from 580 to 410 nm is assigned to the oxyhaemoglobin of the blood. The oxyhaemoglobin of the blood exhibits absorbance maxima in the region 410 to 420 nm. The carbohydrate metabolism in blood can be assigned to the absorption maxima around 350 nm due to NADH and NADPH. These are reduced forms of the co-enzymes for lactate dehydrogenates and 6-phosphate dehydrogenate respectively.

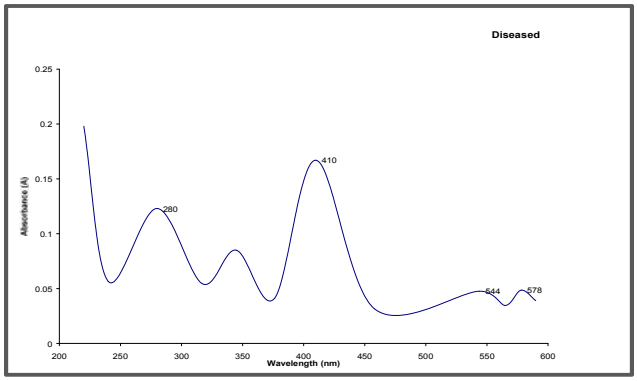

Fig 3 UV spectrum of leukaemia 2 blood

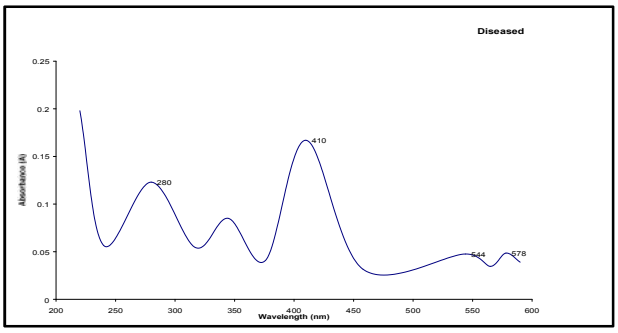

Fig 4 UV spectrum of leukaemia 3 blood

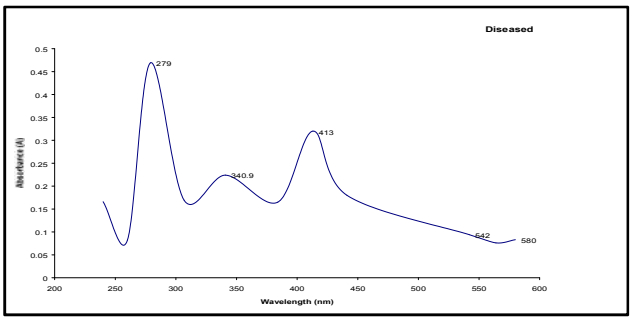

Fig 5 UV spectrum of leukaemia4 blood

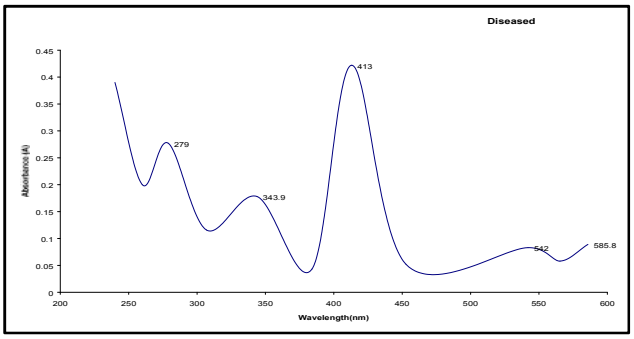

Fig 6 UV spectrum of leukaemia5 blood

The protein metabolism can be conveniently monitored by the changes in the absorbance of wavelength maxima around 280 nm due to tyrosine and tryptophan. The characteristic property of the oxy-haemoglobin exhibiting absorbance in the region 450-420nm has been fixed as the comparison criteria (7). From the table it is evident that there is a decrease in the absorbance maxima of oxyhaemoglobin for the leukemia subjects than that of normal subjects. The change in the absorbance indicates the loss of erythrocytes (or) red blood cells in patients of leukemia. The same blood samples were used to estimate the micro elemental iron using Atomic emission spectroscopy.

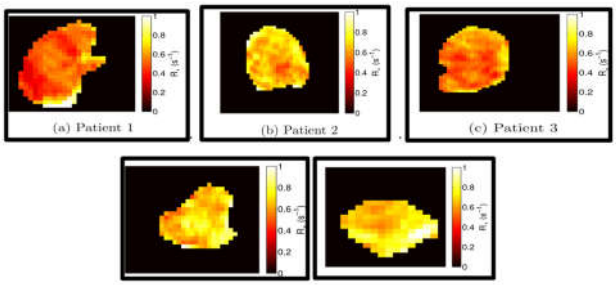

Fig 7 MRI maps for a single slice for all 5 patients

The results obtained show a decrease in the values of the trace elemental iron than the normal values. This decreasing trend of the mineral concentration levels may be due to drugs in taken by the cancer patients. Over half of all cancer patients are anaemic. Anaemia results from red cells aplasia, folate of vitamin B_{12} deficiency and iron deficiency(8). In many instances the cause of anaemia in cancer patients can be due to hypoxia found. In those cases anaemia is described as "Anaemia of chronic disease", probably a remote effect of cancer, where reutilization of home products within the marrow is inefficient (9).

There have been several approaches to reducing hypoxia prior to or during treatment, in an attempt to increase radio sensitivity and to therefore improve outcome. Hypoxia also leads to poor outcomes when using other forms of therapy for treatment. No direct cause has been observed for this reduced sensitivity *in vitro*, but hypoxia is known to select for cells that are defective in the apoptotic response. Impaired effectiveness under hypoxia has been observed for chemotherapy(10) immunotherapy, and photodynamic therapy(11). Overall we conclude that hypoxia results in decreased patient survival.

Of all the methods MRI is more efficient method of imaging. The images produced by MRIs are similar to X-ray images but show more detail of the soft tissues of the body and do not involve electromagnetic radiation. The hypoxia and spatial variation in oxygenation in healthy kidneys is of particular interest to us, as it provides a suitable testing ground for any proposed method to measure tumour oxygenation. The location, size and physiological motion of the kidney during acquisition (primarily due to breathing) make it an ideal test organ for any protocol intended for use in tumours. There is a steep oxygenation gradient between cortex and medulla of the kidney. This gradient is a result of two things: counter current diffusion of oxygen between descending and ascending branches of the vasa recta caused by the particular organisation of vessels in the medulla, and the high metabolic requirements for active solute transport (12). Hence, even healthy kidneys operate with regions close to hypoxia, and the kidneys are therefore susceptible to acute renal injury due to hypoxia in cancer patients. Hypoxia is also linked to the pathogenesis and progression of chronic kidney disease (13).

It is noteworthy from the Fig-7 that the cortex and medulla are distinguishable on the basis of function or microstructure in functional MRI. In MRI, the medulla was seen darker than the cortex because of the difference in amount of oxyhaemoglobin blood oxygenation level dependent contrast measurement were initially developed to monitor blood oxygenation changes in kidney. This method relies on the fact that

deoxyhaemoglobin is paramagnetic, whereas oxyhaemoglobin is not, and so signal can be related to oxygenation status (14). With reference to Fig-7, three of the patients showed regions of significant regions hypoxia (orange region), two patients also showed regions of significant change, but these regions were very small.

CONCLUSION

Overall, the techniques of spectroscopy and Magnetic resonance imaging can provide complementary information regarding blood flow and oxygenation, and that future studies involving should also include further advanced techniques if possible. This is particularly important while we are still ensuring the relationship between the oxygen-enhanced signal change and the underlying tumour biology. Further preclinical experiments in particular would be useful to validate the technique and to enable us to further understand how the various imaging parameters relate to each other and to physiology. After collating the existing cancer data it was found that all cancers produce hypoxiaon tumour cells.

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