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Analysis of Hypoxia in Leukaemia Using UV-Visible, Atomic Emission Spectroscopy and MRI (Magnetic Resonance Imaging)

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ABSTRACT

Hypoxia was identified as a micro environmental component of solid 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 our understanding of how hypoxia influences cellular behavior and therapy response. Furthermore, they have confirmed early assumptions that low oxygenation status in tumours is an exploitable target in cancer therapy. Generally such approaches will be more beneficial to patients with hypoxic tumours, necessitating the use of biomarkers that reflect oxygenation status. Tissue biomarkers have shown utility in many studies. Further significant advances have been made in the non-invasive measurement of tumour hypoxia with positron emission tomography, magnetic resonance imaging and other imaging modalities. In this project, we describe the methods of diagnosis and measuring tumour hypoxia. So this project has been focused to: (a) Diagnose the oxyhemoglobin content of normal blood and cancerous blood by uv-visible spectroscopic technique; (b) Estimate the amount of iron content in cancerous blood patients by Atomic absorption spectroscopy; (c) Diagnosis of the kidney of cancer patients using Magnetic resonance imaging (MRI) for studying the level of hypoxia. Having enumerated the plan of presentation, before going into the details of the above said subdivisions it is of most importance to understand about the components of blood.

Keywords: Leukemia; Blood cancer; Hypoxia

INTRODUCTION

Cancer is the most feared of all diseases. People immediately associate cancer with dying unlike other killer diseases. Cancer usually causes slow death involving pain, suffering, mental anguish, and a feeling of hopelessness. It is the second most common cause of death in the United States and will affect one out of every three Americans during life time [1-21]. According to the U.S Bureau of the census in 2000, 47 people out of every 100,000 died of cancer making it the sixth leading cause of death. Today, 300 people out of every 100,000 will die of cancer, ranking it second. In 1971 the United States declared war on cancer. Since then ten billion dollars have been invested over the years in the cancer research. So intense is the concern to find "the cure for cancer", that more money is collected each year that can actually be spent responsibly on meaningful research. Despite this enormous efforts to combat cancer, the number of new cases of nearly every form of cancer has increased annually over the last century. Cancer research and treatment are extremely complex fields of study because the exact nature of the single cancer cell is elusive. After collating the existing cancer data, it was found that 80-90 percent of all cancers are produced as a result of dietary and nutritional practices, life style (smoking, alcohol etc) chemical, and other environmental factors. This information has now been corroborated by other major agencies; The National Academy of Sciences, The US Department of Health and Human services, The National Cancer Institute, and the American Cancer Society [22-33].

Laboratory tests

Chest X-ray:

In a chest X-ray, electromagnetic energy is used to create images of the internal organs and tissues, including bones. A chest X-ray can show the size, shape, position, and condition of the lungs. Chest X-rays are a routine part of an evaluation for leukemia to assess if the lungs have been affected by the leukemia. Although a chest X-ray exposes the patient to a small amount of radiation, it is less than that experienced in daily living, and the benefits of the image greatly outweigh the slight increase in cancer risk posed by the radiation exposure. However, women who have any chance of being pregnant when the X-ray is taken should tell their doctors, so that special precautions can be taken to minimize the developing fetus's exposure to radiation [34].

Ultrasound of the abdomen:

Abdominal ultrasound uses the same sound-based technology used by depth finders on boats. Using a small device that looks like a microphone, called a transducer or probe, ultrasound waves are directed into the abdomen. These sound waves bounce back to the probe at different rates, depending upon the tissue encountered in their pathway. The returning sound waves are analyzed by a computer to generate detailed images of the organs in the abdomen. In patients with leukemia, ultrasound may be used to evaluate the condition of the spleen and other internal organs that may have been affected by the disease.No preparation is needed for an abdominal ultrasound. In this procedure, which usually takes one to two hours to complete, a technician applies a watery gel to the abdomen and then slides the transducer across the stomach to scan the abdomen from various angles. Most patients say the procedure is painless, although some report slight discomfort from the pressure of the transducer on the stomach. Abdominal ultrasound has no negative side effects and poses no known risk to the body [34].

Computed tomography (CT) scan of the chest:

Computed tomography is a specialized imaging technique that uses X-rays collected from many different angles around the body to generate detailed cross-sectional images as well as three-dimensional images of the body's internal structures and organs, including the heart. Ultrafast CT scans are a special type of CT scan that uses X-rays collected at very short intervals for a more detailed evaluation of the heart in motion. CT scans may be used in some leukemia patients to confirm that the leukemia or its treatment has not affected the heart or lungs.

The procedure is painless and requires the patient to lie as still as possible on a table that is guided into a machine that resembles an enormous doughnut. The machine, called a gantry, directs small doses of electromagnetic radiation toward the body from various angles. Because different tissues of the body absorb varying amounts of radiation, a computer can analyze the radiation transmitted through the body to reconstruct the images of the internal structures and organs. Chest and abdominal CT scans involve exposure to a dose of radiation that typically exceeds the average background dose of radiation we experience in daily living. For most patients, however, the benefits far outweigh the minor risks associated with exposure to this level of radiation [35].

Magnetic resonance imaging (MRI):

A diagnostic test, that uses a combination of large magnets, radiofrequencies, and a computer to produce detailed images of organs and structures within the body.

METHODS AND MATERIALS

Study of oxyhaemoglobin content of normal blood and cancerous blood by uv-visible spectroscopy

Depending on the volume of blood required the proper size syringe is selected. The syringe should be sterile and also dry. If it is not completely dry, red cells will haemolyze in the traces of water present [36]. For safe precaution the syringe may be rinsed with sterile normal saline, taking care that all the saline is expelled from syringe before drawing the blood. Usually one of the veins on the front of the elbow is chosen from the patient's body. The upper part of the arm is constricted by twisting a tournquet of rubber tubing round it, or placing a blood pressure cuff and inflating it to 40 mm Hg. The patient is asked to clench the fist, or to open and close the fist several times, which brings the veins into prominence. When the vein has been chosen and the point of puncture determined, the area is cleaned with spirit or 95% alcohol. The needle should be pushed through the skin firmly and steadily and the required amount of blood is drawn into the syringe. Ideally all hematology tests should be done on fresh blood immediately after it is collected, but this is not always possible especially in spectroscopic experiments. Specimens are collected together from the subjects, and the experiment is carried out. To avoid the spoilage of blood anticoagulant is added and it is refrigerated at 5^{o} centigrade. The anticoagulated blood is centrifuged at 3000 rpm for 10 min. The blood separates into three main layers. The red cell mass settles at bottom of the liquid column, the

plasma settles at the top and a buffy coat in between the plasma and red cell mass [37]. The plasma is removed with the help of pipette and used for experimental purpose. From the table it is evident that there is a decrease in the absorbance maxima 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. Due to the presence of nucleated RBC in the peripheral blood there is a decrease in the total erythrocytes and hence the absorbance. Visible spectrometry has been successfully employed to analyze the samples of leukemia blood. The characteristic property of the oxy-hemoglobin exhibiting absorbance in the region 450-420nm has been fixed as the comparison criteria [38]. The decreasein the absorbance maxima of the cancerous blood than that of normal blood is indicative of the fact that cancer is a degenerative disease. We cannot expect miracle cures just because so much money has been poured into cancer research. At the same time, we should not expect miracles from "cancer cure" facilities that take money from cancer victims desperate to try any treatment in hope of another chance at life. After collating the existing cancer data it was found that all cancers are produce hypoxiaon tumour cells (Tables 1-3).

Table 1: Absorption characteristics of normal blood

Wavelength Maxima (nm)	Formedelements of Blood
580	---
544	----
412	Oxyhemoglobin
344	NADH and NADPH
280	Tyrosine and Tryptophan

Table 2: Absorption characteristics of normal and acute leukemia blood (O^+); Age: 5 years

Sample	Wavelength Maxima (nm)	Formed Elements of Blood	Absorbance (A)
Normal Blood	580	---	0.802
	544	---	0.816
	412	Oxyhemoglobin	1.453
	344	NADH and NADPH	0.818
	280	Tyrosine and Tryptophan	0.941
Acute Leukemia Blood	578	---	0.048
	544	----	0.047
	410	Oxyhemoglobin	0.167
	344	NADH and NADPH	0.085
	280	Tyrosine and Tryptophan	0.123

Table 3: Absorption characteristics of normal and acute leukemia blood (O+); Age: 7 years

Sample	Wavelength Maxima (nm)	Formed Elements of Blood	Absorbance (A)
Normal Blood	580	-----	0.622
	544	-----	0.641
	412	Oxyhemoglobin	1.352
	344	NADH and NADPH	0.854
	280	Tyrosine and Tryptophan	1.045
Acute Leukemia Blood	578	---	0.037
	544	----	0.044
	410	Oxyhemoglobin	0.123
	344	NADH and NADPH	0.058
	280	Tyrosine and Tryptophan	0.102

Study of formed elements of blood by calorimetric method and estimation of the amount of iron content in cancerous blood patients by atomic absorption spectroscopy

The blood samples of acute leukemia subjects of almost same age and blood group were procured from children's' hospital, Egmore, Chennai. India. The blood is drawn and processed using same method as given in previous chapter. CBC method is based on measuring the colour of hemoglobin or a derivative of hemoglobin in a blood sample, the intensity of the colour being directly related to the amount of hemoglobin in the sample. The hemoglobin is converted to acid haematin by the addition of N/10(0.1N) hydrochloric acid, and the resulting brown colour is compared with standard brown glass reference blocks. Acid haematin is insoluble and is present as a colloidal suspension in the fluid. The intensity of the brown colour depends on the amount of acid haematin which in turn, depends upon the amount of hemoglobin in the blood sample. The experiment is performed in the sahli Haemoglobinometer which consists of standard brown glass blocks mounted on a comparator. The hemoglobin content was estimated by the above said method for the subjects exposed to Trinitio toulene and the values were

compared with the normal subjects. Using the above said method the total leukocytes, polymorphs and lymphocytes were also calculated. The values of normal and leukemia blood are tabulated in tables 4 and 5. The iron in the blood is estimated using atomic emission spectroscopy and the results are briefly discussed. The concentration of iron was estimated in the plasma of the diseased subjects by 3410 ICP Atomic emission spectrometer. The results obtained are summarized in the table 6.

Table 4: Blood analysis of normal person by calorimetric method

S No.	Hemoglobin (gms/100ml)	Total leukocyte Count (per cmm)	Polymorphs (%)	Lymphocytes (%)
1	14.5	7200	59	36
2	14.8	7100	57	38
3	14.2	7600	56	38
4	15	6800	60	35
5	15.2	6700	56	37
6	15.5	6600	58	34
7	14.5	7800	62	34
8	15	7800	61	32
9	14.6	8200	65	31
10	14	8100	64	32

Table 5: Blood analysis of leukemia patients by calorimetric method

S No.	Hemoglobin (gms/100ml)	Total leukocyte Count (per cmm)	Polymorphs (%)	Lymphocytes (%)
1	10	12,900	75	28
2	10.5	13,900	70	25
3	11.8	13,600	78	16
4	10.2	13,100	72	23
5	12.5	12,400	72	21
6	11.8	13,500	74	20
7	11.5	10,200	80	15
8	12.8	14,700	78	17
9	12	14,400	76	19
10	11.5	11,400	86	45

Table 6: Trace elemental concentration of acute leukemia blood

S No.	Acute leukemia blood µgms/ml
1	0.832
2	0.632
3	0.785
4	1.301
5	4.578

RESULTS AND DISCUSSION

The analysis of blood samples through calorimetric method reveal that there is a considerable decrease of hemoglobin in the blood of leukemia subjects than normal subjects. There is a considerable increase in the total leukocyte count and lymphocyte percentage. Due to this there is an increase in polymorphs percent value for the leukemia subjects than the normal. Leukocytes are present in the blood in considerably fewer numbers than the RBC. Depending on the type of leukocytes leukemia can be classified as acute, chronic and lymphatic leukemia. In acute leukemia, the total WBC count is usually above 20,000/l and may go as high as 100,000/l. Most of the leukocytes (30-90%) are 'blasts' very immature cells with nucleoli which normally are never seen in the peripheral blood. It may be very difficult to distinguish whether the blast cells are myeloblasts or lymphoblasts. Occasionally the total WBC count may be normal or even below normal with a few immature cells in the peripheral blood. Most of these progress to a more typical leukemic picture in later stage, though that progression may be altered if proper precaution may be taken. The anaemia may also develop due to crowding out of erythroid cells [39-42]. In chronic leukemia the total WBC count may range from 100,000 to 800,000/l. Basophil percentage increases. Hence total leukocyte count increases. There may develop anaemia with this type of Leukemia. The total WBC count in lymphatic leukemia is very high, often in the range of 200,000 - 250,000/ l with 70-90% of the cells being mature lymphocytes. The platelets counts are usually normal or only very slightly reduced. However some patients develop an auto immunise haemolytic anaemia is usually only 1 Leukocyte every 500 RBC. In a normal adult there are about 5,000-10,000 leukocytes per microlitre of blood [43]. For a healthy man the standard value of leukocytes must be

5400 to 9200/cmm. But from tables 4 and 5, it is found that there is a considerable increase in the total leukocyte value than the normal. The results obtained from Atomic emission spectroscopy shows a decrease in the values of the trace element than the normal values. This decreasing trend of the mineral concentration levels may be due to drugs intaken by the cancer patients or may be due to hypoxia. Over half of all cancer patients are anaemic. Anaemia results from red cells aplasia, folate of vitamin B_{12} deficiency and iron deficiency [44]. In many instances the cause of anemia 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 heme products within the marrow is inefficient [45].

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