FTIR & Laser Raman Spectral Analysis Of Cyclophos-phamide And Atomic Emission Spectroscopic Study Of Trace Elements of Cancer

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Abstract:*Cancer is the common term used, by lay people and professionals alike, to designate the most aggressive and usually fatal forms of a larger class of diseases known as neoplasms. Cancer requires long term care and drug therapy, frequently involving the use of multiple drugs. Acute toxic reactions, including food-drug incompatibilities and the effect of vitamin antagonisms have significant clinical outcomes. Cyclophosphamide is one of the widely used drug for treating different types of cancer. Toxic reactions arise due to the drug quality or due to side effect produced by the drugs. The effective use of chemotherapeutic agents in oncology requires a higher level of pharmacologic understanding than any other internal medicine, because the margin for error is slim and the consequences of an error in dosage or dose adjustment may be lethal. The objective of this work is to provide a systematic approach to make a qualitative analysis of cyclophosphamide -anticancer drug, through vibrational spectroscopy and study the trace elemental concentration (Ca, Mg, Zn and Fe) in the blood of the patients administered with it using Atomic emission spectroscopy. We have found that the quality of the sample is good, since the functional groups exhibited the characteristic peak in the appropriate positions in the FTIR and Laser Raman spectra. The analysis of the blood samples of the patients treated with cyclophosphamide reveal that the patients suffer from trace element depletion of Ca, Mg, Zn and Fe. These elements play a vital role in the metabolism of human body. Depletion of these elements causes severe side effects sometimes leads to death.*

Key words: *FTIR, Laser Raman, cyclophosphamide, trace elements.*

I.INTRODUCTION

Leukemia is a disease 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 these 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. It used to be the part of the definition of the disease that it was a 'fatal' condition, that no cure was possible. However, with the advent and increasing use of powerful chemotherapeutic drugs, increasing number of patients with leukemia, treated and are possible to live for few more years. But the side effects of the drug have adverse effects on them.

Safe drug use requires a choice of an appropriate route and schedule of administration, a determination of safe dosage range, an awareness of routes of drug elimination and an adjustment of dose to accommodate organ dysfunction, an awareness of the incidence and course of potentially life threatening toxicity and a knowledge of drug interactions as influenced by dose and schedule to maximize favourable interactions and minimize toxicity (1). Each drug is prepared by standard methods. The main function of the drug is to cure the disease. So it is of great importance to know in which manner a drug is absorbed in the human body and its effects.

Drug action occur in two stages in the body. They are, the pharmaceutical stage (dissolution or disintegration of the drug) and the pharmacodynamics stage (the body's physiologic or psychological response to a drug or combination of drugs).Of this stage pharmacodynamics stage is of great importance, due to fact that some drugs may induce adverse effects. Those drugs are called as cytotoxic drugs. Majority of cytotoxic drugs have more profound effect on rapidly multiplying cells. Their most important target of action are nucleic acid. This may be due to chemicals present in the drug or due to their inferior quality. Since Cyclophosphamide falls under the category of high cytotoxic drugs our attention was turned towards the qualitative analysis and estimation of trace

elements in the blood of diseased subjects administered with it.

Cyclophosphamide is the most widely used alkylating agent (2), was synthesized in the belief that it would be inactive in the body until its ring structure was broken down by an enzyme, more common in cancer cells than in normal cells. It was thought that cyclophosphamide would be inert until it penetrated the cancer cells, where it would be converted to the active derivative, thus damaging the cancer cell . This supposition did not turn out to be correct however. The structure of the drugs is presented in the figure 1.In the body, cyclophosphamide is converted to the active compound mainly in the liver rather than in the tumour (3,4) . Because it must be enzymatically oxidized with cleavage of the N-P bond to give an active metabolite, it is not active in vitro. It is converted to 4- hydroxy cyclophosphamide and aldophosphamide. It is chemically unstable undergoing conversion to acrolein (CH_2=CH-CHO) and phosphoramide mustard.Despite an incorrect premise concerning its actions, cyclophosphamide is superior to other alkylating gents. It is frequently used to treat leukemia and lung cancer. Cyclophosphamide may be given by mouth even though the absorption is incomplete, however it is often used intravenously to ensure maximum effectiveness. It may cause nausea and vomiting hair loss, testicular atrophy, ovarian fibrosis and suppression of menstruation,bladder irritation and bleeding Hemorrhagic cystitis There is a risk of developing a secondary blood cancer such as leukemia or myelodysplasia after taking cyclophosphamide.

II EXPERIMENTAL WORK

Cyclophosphamide with spectroscopic purity was procured from appropriate sources. The fourier transform infrared spectra of this compound was recorded over the region 400-4000 cm^{-1} using BRUKER IFS 66v FTIR spectrometer and Laser Raman spectra was analysed in the range 10-4000cm^{-1} using BRUKER IFS 106 RAMAN MODULE Spectrometer The spectra are presented in figs 1, 2 and the vibrational band assignments are summarized in table 1. All sharp bands observed in the spectra are expected to have an accuracy of ±1 cm^{-1}.

Spectra and qualitative analysis

The fabrication and production of life saving drugs play an important role in our daily life. The purity of the drugs become very important. Hence, during the fabrication of the drugs , various raw materials that are available for the fabrication should have to undergo a qualitative test. Infrared and Raman spectroscopy produce a wealth of information about the quality of the sample(5-10).

Nutrition and Cancer

Drug nutrient interactions are commonly overlooked aspects of the prescribing practices of physicians. Therapeutic effects or side effects of medications may ultimately diminish the nutritional status, or conversely, the nutritional status of the patient may decrease the drug efficacy or increase its toxicity. A number of elements are present in very small amounts in the tissues and blood that are essential to growth, health and development . This subdivision throws light on the risk factors of nutrition for the acute leukaemia patients administered with cyclophosphamide (11).

The necessary nutrition in the form of minerals such as calcium, magnesium, zinc and iron are estimated using atomic emission spectroscopy and the results are briefly discussed. The blood samples of acute leukaemia subjects of almost same age and blood group, and same dosage of the drug were procured. The concentrations of calcium, magnesium, zinc and iron were estimated in the plasma of the diseased subjects by 3410 ICP Atomic emission spectrometer. The results obtained are summarised in the table 2.

The results obtained shows a appreciable variation in the values of the trace elements and minerals than the normal values. This decreasing trend of the mineral concentration levels may be due to drug intaken by the patients. The interaction between drugs and nutrients has become known during the past few years. Some of these drug interactions involve the absorption or utilisation of minerals of the body and ultimately compromise a patients health. A cancer patient who is taking anti cancer drugs should be aware that his or her diet may have to be supplemented with the indicated trace elements (12). Since anti cancer drugs are taken for months or years together vitamin supplementation should be instituted because, these drugs produce undesirable effects on patients. The reasons for the study of minerals such as calcium, magnesium, zinc and iron are as given below.

Calcium.

The values of calcium are slightly high in majority of the patients as shown in table 2.This is one of the side effects of cyclophosphamide. Cyclophosphamide (a component of Revimmune, Cytoxan, Neosar, and other drugs) suppresses bone marrow growth, can induce early menopause in addition to stimulating osteoclasts. The more often, loss of bone mass is due to cancer treatments themselves. High blood calcium levels are called hypercalcaemia. This usually happens in cancer patients taking cyclophosphamide.The cancer makes calcium leak out into the bloodstream from the bones, so the calcium level in the blood gets too high. Cyclophosphamide fails to control the calcium leakage in the blood. The cancer may also affect the amount of calcium that kidneys are able to get rid of (13). Damaged areas of bone can release calcium into the bloodstream. If this is not treated with proper calcium supplements cancer patients along with their disease they face the following problems.

Dehydration from being sick a lot or having diarrhoea may also increase calcium levels. If not treated, high blood calcium can make the person more drowsy until, are sleeping nearly all the time. If still not treated, that may lead into a coma and eventually die. The types of cancers that are most commonly associated with high blood calcium areleukaemia ,lung cancer and breast cancer. .Because calcium plays a role in the normal working of the brain and spinal cord, patients with severely high calcium levels may alsohave fits (seizures)be unable to coordinate muscle movement, which can affect walking, talking and eating (14).

Magnesium

Magnesium is found everywhere in our body and half of our total magnesium is found in our bones. Although our blood only contains about 1 percent of our total magnesium, it carries the other 99 percent through our cardiovascular system to be stored in tissue, bone and organs. There is a power and a force in magnesium that cannot be equalled anywhere else in the world of medicine. There is no substitute for magnesium in human physiology; nothing comes even close to it in terms of its effect on overall cell physiology. Without sufficient magnesium, the body accumulates toxins and acid residues, degenerates rapidly, and ages prematurely. It goes against a gale wind of medical science to ignore magnesium chloride used transdermally in the treatment of any chronic or acute disorder, especially cancer (15).

Because cyclophosphamide is indiscriminate in killing cells, it often comes with unwanted side effects. Cancer patients receiving cyclophosphamide may be at high risk for developing hypomagnesemia due to side effects such as diarrhea that can deplete magnesium stores, according to Dr. Muhammad Wasif Saif, MD, MBBS in "Supportive Oncology." cyclosporine can decrease magnesium levels to a point of deficiency. The deficiency can occur within three weeks of beginning chemotherapy treatment and can last for months. The concentration of magnesium for the patients taken for study shows (table 2)deficiency which is one of the major side effects of cyclophosphamide (16). To avoid this patients should have regular blood tests that reveal levels of magnesium as well as other essential nutrients. IMagnesium abundant foods or supplement must be prescribed to the patients. Good sources are seeds, nuts, legumes and unmilled cereal grains as well as dark green vegetables, in which magnesium is essential constituent of chlorophyll.

Zinc

A large body of evidence suggests that a significant percentage of deaths resulting from cancer could be avoided through greater attention to proper and adequate nutrition. Although many dietary compounds have been suggested to contribute in the prevention of cancer, yet there is a strong evidence to support the fact that zinc, a key constituent or cofactor of over 300 mammalian enzymes, may be of particular importance in host defence against the initiation and progression of cancer. Remarkably, 10 per cent of the U.S. population consumes less than half the recommended dietary allowance for zinc and is at increased risk for zinc deficiency. Zinc is known to be an essential component of DNA-binding proteins with zinc fingers, as well as copper/zinc superoxide dismutase and several proteins involved in DNA repair. Thus, zinc plays an important role in the functions of transcription factor, antioxidant defense system and DNA repair. Dietary deficiencies in the intake of zinc can contribute to single and double-strand DNA breaks and oxidative modifications to DNA that increase risk for cancer development. zinc deficiency could disrupt the function of both signaling molecules and proteins directly involved in DNA replication and repair. Limited availability of cellular zinc due to zinc deficiency could result in a loss of activity of these zinc-dependent proteins involved in the maintenance of DNA integrity and may contribute to the development of cancer. Zinc deficiency has also been shown to upregulate expression of the tumour suppressor protein, p53, Zn concentration decreases in cancer patients . It is believed that low concentration of plasma Zn in cancer patients is due to the increased requirement of Zn by cancer tissues . This

seems to be reasonable because of the fact that tumor cells have high rate of DNA synthesis and most of the enzymes involved in the nucleic acid synthesis are Zn dependent (17)

There is evidence to suggest an intriguing link between zinc and cancer. In *in vivo* studies, it has been shown that zinc treatment increases resistance against tumour challenge in mice and decrease the incidence of spontaneous lung tumours arising in mice. It is clear from literature that zinc is of extraordinary and diverse importance in cancer biology. By prescribing either supplements to patients administered with cytotoxic drugs the effect of cancer can be reduced. Meat, fish milk and milk products can be prescribed to take in large amounts for the patients.

Iron

Over half of all cancer patients taking cyclophosphamide are anaemic. Anaemia results from red cells aplasia, folate of vitamin B_{12} deficiency and iron deficiency. In many instances the cause of anaemia in cancer patients can't be 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 . Such patients have low erythro protein levels (18).Due to lack of iron, cells replication stops and the cell dies. This leads to the deficiency of iron which in turn results in anaemia. It has been shown that iron complexes can initiate the production of free radicals and that excess stores of iron can be best supplied by shellfish, Kidney, Meat poultry, eggyolks and cereals.

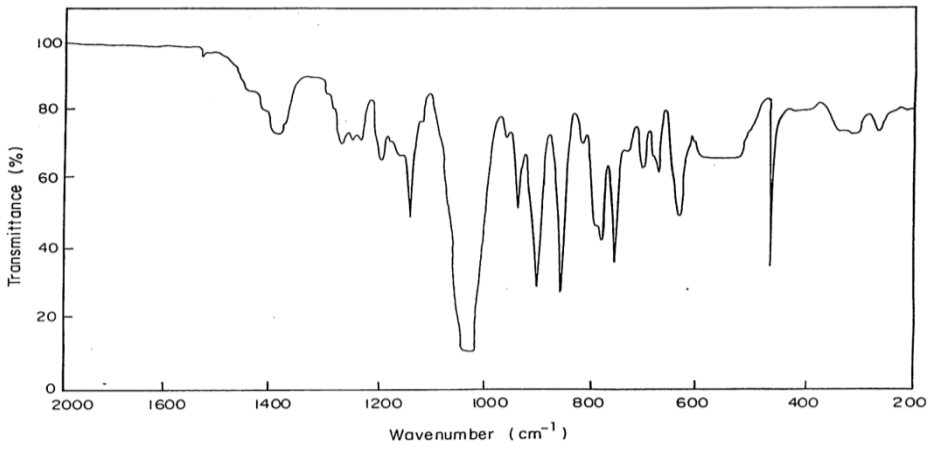

Figure .1 Infrared Spectrum of Cyclophosphamide

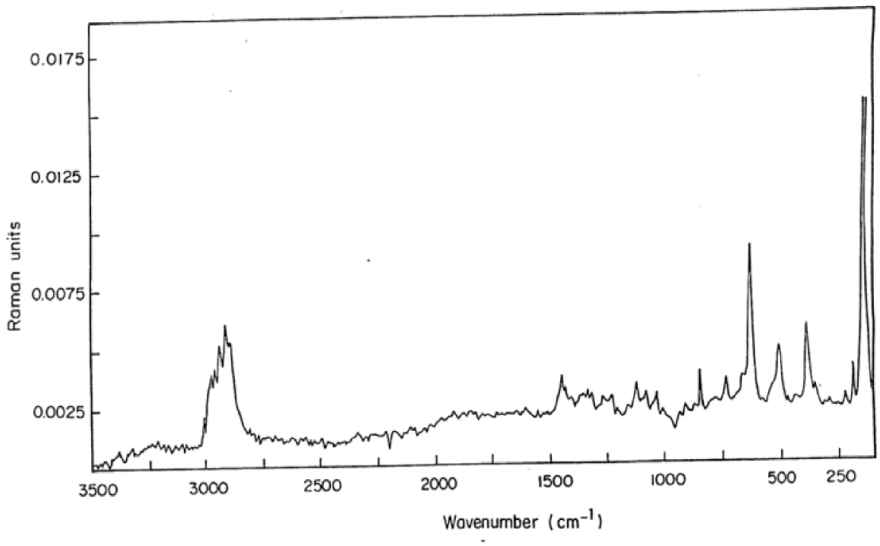

Figure .2 Laser Raman Spectrum of Cyclophosphamide

TABLE 1

FTIR and LR Spectra And Vibrational band investigations on Cyclophosphamide

Frequency (cm^{-1})		Description
FTIR	LASER RAMAN	
---	397 (w)	Aromatic out of plane ring deformation
450(vw)	----	Aromatic out of plane ring deformation
490(vw)	-----	Aromatic out of plane ring deformation
---	515(ms)	Aromatic out of plane ring deformation/C-Cl stretching
---	637 (vs)	Aromatic in of plane ring deformation
660(ms)	---	Aromatic in of plane ring deformation
----	739 (mw)	Aromatic in of plane ring deformation
760 (w), 820 (w)	----	C-Cl Stretching
860 (mw)	----	C-Cl Stretching/P-O-C stretching
900(ms)-970(ms)	----	P-O-C stretching
1028(ms)-1100(s)	1038 (w)	C-H in plane deformation
1130(w)-1230 (vs)	1123(w)- 1275(w)	C-H in plane deformation/P=O stretching
1330(mw)	1347(mw)	C-N stretching/P=O stretching
1410(vw)	---	CH_2/P=O stretching
----	1460(w)	CH_2/P=O stretching
1580(w)-1780(w)	--	Aromatic C-C stretching
----	2916(vs)- 2971(vw)	Aromatic C-H stretching
	3221(vw)	N-H stretching

TABLE -2

Atomic emission spectroscopic studies on trace elements of acute leukaemia blood treated with cyclophosphamide

Elements	Concentration of trace elements (µgms/ml)										
	Normal values	Acute leukaemia values									
		A	B	C	D	E	F	G	H	I	J
CALCIUM	100	158.004	167.449	187.116	180.437	123.125	118.002	97.449	175.113	156.089	133.045
MAGNESIUM	30-45	13.230	16.004	17.800	23.005	19.130	16.335	18.550	11.312	31.887	29.520
ZINC	0.9-1.1	0.549	0.448	0.553	1.849	0.937	0.486	0.738	0.854	0.635	0.386
IRON	10	0.832	0.632	0.785	1.301	4.578	3.556	7.543	5.344	3.857	2.558

Conclusion

Though studies that have been done and ones that are ongoing, we can discover what combinations of nutrients can be used to prevent cancer. Moreover cancer patients must be counseled about food drug interactions, all medications should be reviewed for potential effects. A diet history in relation to ascertain the use of alcohol, vitamin and mineral supplements, and other supplements when determining the extent of drug nutrient interactions, it is also important to consider patients age, drug dose and duration of therapy or certain medications.

Despite of taking proper actions for treatment of cancer, it is each and every one's responsibility to learn about the risk factors involved in cancer development and then modify those risk factors accordingly. In order to prevent

cancer a proper plan should be devised for each individual. Children should be taught of the risk factors modifications. Obviously there are some risk factors like air and water, that cannot be controlled, but it necessary for the human community to devise a plan to modify environmental risk factors. The other risk factors can be controlled, eating a prudent diet, avoid known cancer-causing chemicals and take rotational amounts of certain vitamins and minerals. The increase and incidence of cancer can be reduced by eating the right foods and taking the right amount of vitamins and minerals shown to have anticancer and anti- oxidant effects, and shown to be needed for the immune system to function well. Enough new data have emerged in recent basic clinical and epedemiological studies to justify for the hypothesis that micronutrients may prevent the initiation or development of cancer. Hundreds of potential chemo preventive compounds have been identified from dietary sources. A single pill simply will not do the job. Begin by making changes in your life style and diet, by not smoking or drinking alcohol, and also it is important for dietitians to monitor in hospital drug therapy as well as discharge medications, to provide appropriate counseling and to document the patients comprehension in the medical record.

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