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Case - Control Study of Blood Samples of Cancerous Brain Tumor Patients by ICP - OES

Shuchi Srivastava

Saha's Spectroscopy Laboratory, Department of Physics, University of Allahabad, Allahabad 211002, India
Email: *srivastava.shuchi27[at]gmail.com*

Abstract: ***The blood of the four brain tumor patients and four healthy donors have been analyzed for the selected trace metal such as cadmium, copper, chromium, iron, zinc, nickel and lead by ICP - OES and develop a correlation between these metal in cases and control. The case - control study shows that trace metal like copper, iron and zinc may influence the carcinogenic process while the metals cadmium, nickel and lead are not traceable neither in cases nor in control. The study reveals that the level of the copper and iron are high in the cases than control. The suspected cause is due to these elements promote angiogenesis and metastasis process. The metal chromium is observed in the cases while it was not detected in any sample of controls. The major goal of our study is the development and description of a routine methods for the analysis of human biological samples by ICP - OES because biological samples are limited in the literature or studies are only dealing with a few toxic or essential trace elements and the study shows that the concentration and correlation of these toxic metal support the angiogenesis and metastasis processes.***

Keywords: Case - control study, Angiogenesis, Metastasis, Correlation, minimally invasive technique, Poor prognosis and ICP - OES

1. Introduction

Cancerous brain tumor disease is a complex health problem in which environmental, dietary, geographical, demographic and genetic factors contribute [1, 2]. Trace metal plays a significant role in balancing body. Carcinogenic trace metal disturbs balance of the body which includes DNA damage, mutagen, generation of free radical, DNA protein cross link, chromosomal aberration and pathological proliferation of cells, lipid per oxidation, apoptosis, cellular toxicity and inappropriate activation of cellular signaling pathways. These metals generate free radical which is highly reactive and generate mutation like DNA strand break, DNA protein crosslink [3, 4]. Although, metallic species participate in the carcinogenic process use as a potential marker for tumor differentiation or cell proliferation, or as a predictor of poor prognosis remains unclear because of a complicated mechanism of the action of trace elements on molecular level. The determination of trace elements in blood of brain tumor patients may give information about the formation of brain tumor. The deficiency or abundance of metallic elements give rise many clinical and pathological disorders in humans. Since the air, water, soil and food stuffs are becoming polluted day by day due to industrialization, urbanization, sewage waste and agricultural practices, therefore concentration of these elements is expected to be altered from their normal condition. After ingestion, heavy metals associated with hemoglobin and whole blood is useful for monitoring occupational exposure to contaminants such as trace toxic metal.

In recent year, there are several advance technological methods of cancer testing that have been developed and are continuously being improved. Some of these methods include: circulating tumor cells (CTCs), cell free DNA (cfDNA), next - generation sequencing (NGS), immunohistochemistry (IHC), genomic profiling, multiplexed immunofluorescence (MIF), digital pathology, artificial intelligence (AI) in pathology, metabolomics and proteomics etc. are clinical method [5, 6]. These clinical methods play crucial roles in cancer diagnosis, prognosis, treatment selection, and research, contributing to the advancement of precision oncology and personalized medicine.

Schicha et al [5] studied human brain tumor by neutron activation technique (NAA) and detected trace elements like Co, Fe, Rb, Se, Zn, Cr, Ag, Cs, Sb and Sc. Lowndes [6] examined the role of copper in tumor angiogenesis while Dimitropoulou et al [7] investigated the amount of zinc intake in brain cancer using case control study. Recently Arslan et al [8] investigated the levels of the elements: Cd, Fe, Mg, Mn, Pb and Zn by using AAS and were found significantly elevated in the patients as compared to control. In addition, the concentration of Cu was found to be decreased [8]. The comparative study of elemental analysis techniques reveals that in most of the studies Atomic Absorption Spectroscopy (AAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) technique is used. The AAS technique has ability to measure accurately many analytes at trace levels in the range of sample matrices. However, relatively long analysis time, limited multi - elemental capability and complex; analyte dependent sample preparation requirements have leads analytes to investigate the specimen by inductively coupled plasma - optical emission spectroscopy (ICP - OES) for their applications. The scrutiny of the literature reveals that ICP - OES method better in comparison to other clinical techniques because of minimally invasive method and poor prognostic value and control risk of metastasis. ICP - OES is capable of detecting trace level of elements in samples because of high sensitivity, accuracy, multielement, rapid and quantitative analysis. But there are some limitations such as sample preparation and cost in comparison to other clinical method. Therefore, the ICP - OES is a fast multi - elemental detection technique and is gaining popularity in clinical field due to its sensitivity, rapidity, accuracy, reproducibility and cost effectiveness. Due to these supremacies of ICP - OES, author decided to access the concentration of selective carcinogenic metals in the blood of brain tumor patients and compared with healthy persons.

2. Methods

Sample Collection - The samples were collected from admitted patients of Department of Surgery, Motilal Nehru Medical College, Prayagraj, India. The study was performed on 8 patients, in which 4 cases were of brain tumor and 4 patients were of control (without tumor). All the patients were from same geographical area. The blood samples were extracted during surgery. The samples were collected from antecubital vein by using appropriate precautions to prevent contamination with exogenous trace elements by trained surgeon. The blood samples were collected in heparinized evacuated tubes. After sampling, samples were stored at a very low temperature in a zip mounted high density polythene bag duly labeled with relevant codes related to the donor's name, age, gender, eating and drinking habits and social and general health status. All the records were compiled on regular proforma at the time of sampling and make uniform sample by shaking it by hand.

Importance of Sample - After ingestion, heavy metals often become predominantly associated with hemoglobin and for this reason whole blood is particularly useful for monitoring occupational exposure to contaminants such as trace metal. Blood is the transport medium for nutrient and trace metal. In general, it is complex matrix so it is predigested. ICP - OES is used because it is fast and multi - element detection technique. Therefore, it gives rapid and reliable information about the trace metal in blood.

Sample digestion - For digestion of blood samples, method established by NIOSH was used. This method was used to obtain lower detection limit and/or to improve accuracy, precision and selectivity. An accurately weighed 10 gm blood was transferred to a beaker. After that, 10 ml digestion acid (which consisted of three volume of conc. HNO_3 with one volume of conc. $HClO_4$ and one volume of conc. H_2SO_4) added to each blood sample. Then it was heated at 110^0C for 2 h. Later the hot plate temperature was increased to 250^0C and heated until 1 ml blood remained. Then the hot mixture was allowed to cool. The content of beaker was transferred to a volumetric flask of 25 ml and then diluted to the mark with deionized water. The both type of digested samples of controls and cases were treated in the same way to allow investigating relative differences in the distribution of metal containing species between the cancerous tissue and healthy tissue blood. Quantitative analysis was performed by ICP - OES with automatic background compensation and under optimum analytical conditions. The plasma of the samples was analyzed for seven metals; Cr, Cu, Cd, Fe, Zn, Ni and Pb. The samples were treated and run separately on the ICP spectrometer.

ICP - OES analysis - Cd, Cu, Cr, Fe, Zn, Ni and Pb in digested blood samples of cases and control were determined using ICP - OES. Regarding the spectral lines, the analytical wavelengths monitored for each element are presented in Table 1 and 2. The amount of Cd, Cu, Cr, Fe, Zn, Ni and Pb were quantified based on standard calibration curves and intensities of each element.

3. Result and Discussion

ICP - OES performance - ICP - OES is capable of detecting trace level of elements in samples because of high sensitivity, accuracy, multielement, rapid and quantitative analysis. Therefore, ICP - OES method could be further developed into a routine diagnostic or screening tool. This would involve standardizing the procedures for sample collection, preparation and analysis to ensure consistency and reliability in clinical setting. But there are some limitations such as sample preparation & cost. The case – control study reveals that the presence of carcinogenic toxic trace metal such as Cd, Cu, Cr, Fe, Zn, Pb and Ni of cancerous blood of brain tumor patient diagnosed using ICP - OES and also shows the correlation between these toxic metals. Before the analyzing the carcinogenic toxic trace metal in ICP - OES, we use the NIOSH digestion method because of high complex matrix of blood sample.

Role of trace metal in tumor formation - The obtained results are given in tables 1 and 2. The case - control study shows that trace metal like copper, iron and zinc may influence the tumor formation process. By monitoring changes in trace metal distribution concentration in tumor and its role in the formation of tumor can be understood. The scrutiny of the results obtained reveals that the level of copper and iron are higher in cases than control. The suspected cause due to this, promote angiogenesis process. The metal chromium is observed in the cases while it is not detected in any sample of controls. The relationship of dietary elements is complex and it is not possible to separate the effect of one element alone from the effects of other. However, these studies have to be further verified in large number of samples.

Correlation between toxic trace metal - The correlation study of the blood sample of cases and control is also studied. The obtain result shown in the table 3 and 4. The study shows that the correlation between Cu and Cr is 0.545857, Fe and Cr is 0.365383, Zn and Cr is 0.834623 in the cases. But Cr is not detected in the control. Also, in the cases correlation between Fe and Cu is 0.583677 but in the control the correlation between Fe and Cu is - 0.13564. The correlation between Cu and Zn in the cases is 0.888558 but, in the control value is - 0.62003. So, the result shows that the process of angiogenesis and metastasis is increased by the Cu, Zn and Fe. Because in the cases it shows the significant correlation. The level of these biomarker compared in the cases and control so biomarkers is an important tool in the study of environmental health and clinical toxicology.

4. Conclusions

The present study demonstrates the capability of ICP - OES technique for the trace metal analysis of biological samples. The study found notable differences in element concentrations between brain tumor patients and the control. Since biological samples act as an indicator of environmental exposure & contribute further for investigation of specific exposure response relationship in the form of disease. The case - control study shows that Cu, Fe and Zn play an important role in angiogenesis and metastasis process and Cr is absent in the control. The correlation study performed by MS EXCEL DATA ANALYSIS TOOL PAK. For the accuracy and precision of the result large number of samples is required.

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Table 1: Concentration of trace elements in blood sample of cancerous brain tumor cases

Patient & Age	Description	Cd (mg/g)	Cr (mg)	Cu (mg/g)	Fe (mg/g)	Ni (mg/g)	Pb (mg/g)	Zn (mg/g)
Patient 1 (50)	Low grade Glicora (Brain tumor)	NT	8.049	7.244	0.195	NT	NT	4.655
Patient2 (45)	Glioblastoma (brain tumor)	NT	1.870	5.940	0.237	NT	NT	4.001
Patient 3 (70)	Glioma (Brain tumor)	NT	2.423	5.729	0.052	NT	NT	4.230
Patient 4 (3)	Epiclormoidai (Spinal cord tumor)	NT	1.945	0.079	0.065	NT	NT	3.591

NT: Not traceable

Table 2: Concentration of trace elements in blood samples of control

Patient & Age	Description	Cd (mg/g)	Cr (mg/g)	Cu (mg/g)	Fe (mg/g)	Ni (mg/g)	Pb (mg/g)	Zn (mg/g)
Patient1 (40)	Chest injury	NT	NT	1.051	0.175	NT	NT	4.581
Patient 2 (60)	Duodenal perforation	NT	NT	4.272	0.051	NT	NT	3.532
Patient3 (35)	Intestinal obstruction	NT	NT	6.278	0.245	NT	NT	3.976
Patient4 (30)	Tubercular meningities	NT	NT	6.939	0.062	NT	NT	3.922

NT: Not traceable

Table 3: Correlation between traceable elements of blood sample of cases

	Cr (mg)	*Cu (mg/g)*	*Fe (mg/g)*	*Zn (mg/g)*
Cr (mg)	1			
Cu (mg/g)	0.545857	1		
Fe (mg/g)	0.365383	0.583677	1	
Zn (mg/g)	0.834623	0.888558	0.387429	1

Table 4: Correlation between traceable elements of blood samples of control

	Cu (mg/g)	*Fe (mg/g)*	*Zn (mg/g)*
Cu (mg/g)	1		
Fe (mg/g)	- 0.13564	1	
Zn (mg/g)	- 0.62003	0.541552	1

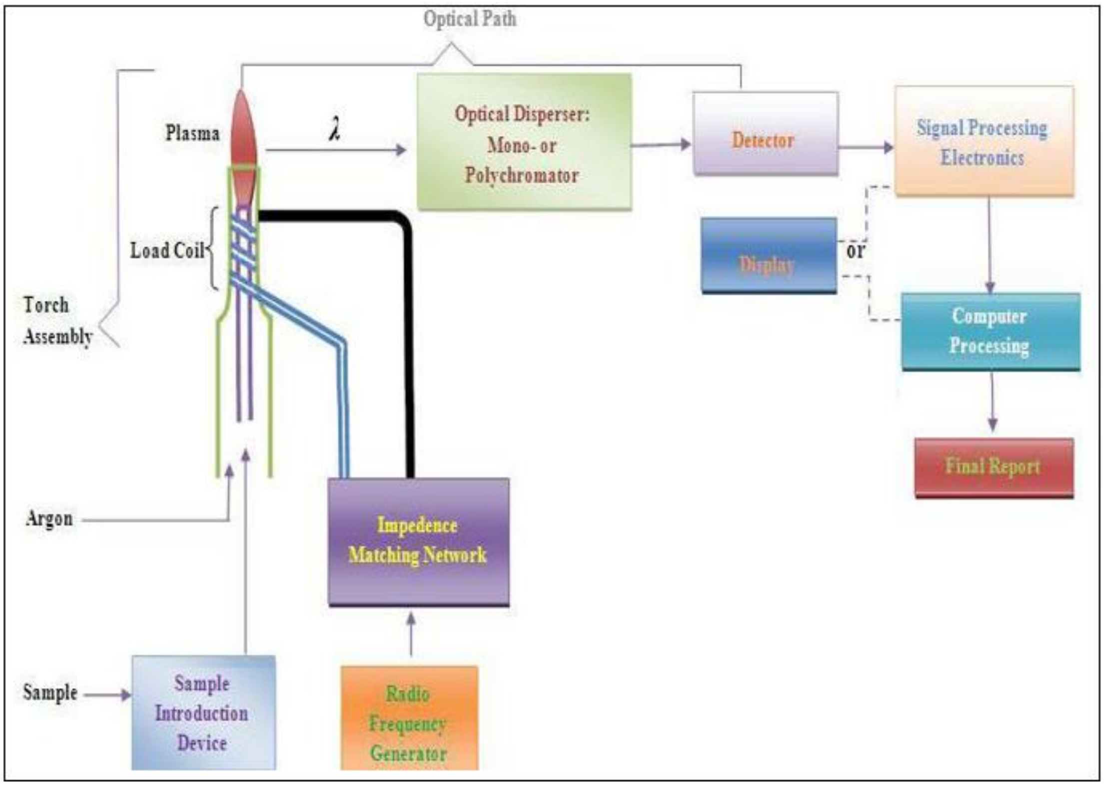

Figure: Inductively coupled plasma optical emission spectroscopy