EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 6 (June-2015) pp. 380-387

www.ijcrar.com

Determination of copper and lead in serum of patients with acute leukemia

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KEYWORDS

Acute leukemia,
Lead in serum,
DPAdSV,
ICP-OES,
Cu,Pu.

A B S T R A C T

The relations between malignant haematoloogical diseases and trace heavy metal in blood have not been understood clearly. Alterations in serum Cu levels may frequently occur in many neoplastic diseases, including leukemia. Therefore, it is necessary from an analytical point of view to develop sensitive and economical methods for the determination of trace amount of heavy metals and the relationship between changes in concentrations of these metals and the development of hematological malignancies. Differential Pulse Stripping Voltammetry (DPAdSV) is relatively inexpensive and is one of the most sensitive and selective techniques in the determination of trace amounts of metals. The levels of heavy metals such as, Cu and Pb were determined in the serum of 20 50 patients with acute leukemia before initial chemotherapeutic treatment and compared to 15 30 apparently health control group using two different analytical techniques; (DPAdSV) and inductively coupled plasma optical emission spectrometry (ICP-OES). The selection criteria for the patients and controls were the lack of recent blood transfusion history and taking any medication with mineral supplement. The Serum levels of Cu was significantly lower with acute leukemia than in controls (p=0.003), while Pb was insignificantly elevated (p=0. 381). Conclusion: In this study, we found the levels of Cu to be lowered and of lead to be elevated in patients with acute leukemia. Further studies are needed to clarify the role of these elements in pathogenesis of acute leukemia. And also a comparative study was carried out between the results using DPAdSV and ICP-OES techniques, which are in very good agreement.

Introduction

Acute leukaemia is a clonal malignant disorder affecting all age groups. It is characterized by the accumulation of immature blast cells in the bone marrow. This results in bone marrow failure, reflected by peripheral blood cytopenias and circulating blast cells. In most cases the etiology is not obvious, but internal and external factors associated with damage to DNA can predispose to acute leukaemia (1).Blood is the transport medium for the nutrients and trace metals to and from the

tissues and, therefore, provides rapid and reliable information about the trace metal metabolism in human body (2,3).Several studies have been reported in the recent years regarding the trace metal evaluation in the body liquids but because of natural significance and ease of sampling, blood is the most commonly used specimen. Consequently, whole blood, serum and plasma have been used in biological research for the determination of trace metal status of individuals and groups (4-6).

Copper is an economically important element that is found in only trace quantities in the Earth's crust. For both plants and animals it is required as a trace nutrient, but excessive amounts are toxic (7). High amounts of copper in the human body can cause stomach and intestinal distress such as nausea, vomiting and diarrhea. Copper and zinc have been associated with normal lymphocyte maturation and regulation of immune function. Low levels of these minerals have been demonstrated in a variety of dysfunctions of the immune system (8).Serum concentrations of copper and zinc are modified in some cancers; serum copper concentrations may be increased in some leukemias (9), and lymphomas (10), The levels of Cd and Pb in the human body have a great toxicological significance being responsible for a number of health impairments.

Lead is known to be a toxic metal that accumulates in the human body throughout the lifetime. Its cumulative poisoning effects are serious hematological damage, brain damage, anemia, and kidney malfunctioning (11). Imbalances in the optimum levels of these trace elements may adversely affect biological processes and are associated with many fatal diseases, such as cancer. There are several reports on serum trace element levels in malignant diseases including leukemia and lymphomas (12). But, there are contradictory data between the previous studies, done related to the trace elements state in acute leukemia (13).

Material and methods, patients and control, study subjects

This study included 50 patients were newly diagnosed as acute leukemia (20 females and 30males) aged between 20 and 50 years. Diagnosis of acute leukemia based on symptoms, physical finding as well as complete blood picture with total and differential leucocytic count, bone marrow aspirate and immunophenotyping when needed. All the patients were enrolled in the study before receiving any chemotherapeutic agents. The selection criteria for the patients and controls were the lack of recent blood transfusion history and taking any medication with mineral supplement. The patients were recruited from clinical hematology unit, Internal Medicine department, Assuit University Hospital. The control group consisted of 30 healthy subjects (14 females and 16 males) aged between 20 and 45 years were chosen for our study and approved by the Ethic Committee.

Sample collection and processing

Blood samples (5 ml) of patients were collected by venous puncture. Blood samples of control were collected from the same areas of patients.. The puncture site was cleaned to remove any expected contamination before sampling. Separate and disposable sterilized plastic syringes were used for blood collection. The blood sample was left standing for 1h to coagulate; serum was separated at 3500 rpm centrifugation for 10min, transferred to 5 ml polystyrene tube, and stored at -5 ºC until analysis.

Reagents and solutions

All reagents are of analytical grade. Solution of each Cu(II) and Pb(II) were prepared respectively by dissolving the required amounts $Cu(NO_3)_2.2H_2O$ and $Pb(NO_3)$ in bidistilled water.

Experimental instrumentation

All glassware was soaked in 10% (v/v) HNO_3 for 24 h and rinsed three times with distilled water and then in redistilled water before use:

- Anodic differential pulse stripping voltammograms were recorded by polarographic Analyzer stripping voltammeter Model 264 A (EG&G, Princeton Applied Research; Princeton, NJ, USA), coupled with a PAR 303 A Static Mercury Drop Electrode (SMDE; drop size: medium, area of the drop: 0.014 cm2). The polarographic cell bottom (PAR Model K 0060) was fitted with Ag/AgCl saturated KCl, reference electrode, and platinum wire used as a counter electrode. A PAR 305 stirrer was connected to the 303 SMDE. A PAR Model RE 0089 X-Y recorder was used for recording the voltammograms. Before measurements the sample solution was deaereated by bubbling for 16 minutes with nitrogen. During measurements, an inert atmosphere over the solution was maintained by flushing with nitrogen. During the deposition step, the solution was stirred automatically, followed by a quiescent period of 15 sec before scanning.

- All the determinations were carried out by inductively coupled plasma optical emission spectrometry (ICP-OES). Thermo Fisher Scientific Announces Enhanced iCAP 6200 Optical Emission Spectrometer was used with the following operating conditions: Nebulizer Gas flow rates: 0. 6 l/min; Auxiliary Gas Flow: 0.5 l/min; Coolant Gas Flow: 12 l/min; Nebulizer Argon Flow: 0.6 l/min; Pumb Speed: 45 rpm; RF Forward Power: 1150 Prior to analysis.

- pH was measured with Hanna microprocessor pH model 211.

Sample digestion

One milliliter serum sample was wet digested in covered glass beaker containing a 10mL (1:1) HNO_3 / $HClO_4$ acid mixture. The digest was transferred in to a 25 mL pre-cleaned measuring flask, diluted to the mark with double distilled water, and stored for analysis. Blank solution was treated and prepared in the same way as the samples. Each sample and each blank were prepared in triplicate.

Analytical procedure

The following parameters were used to perform Differential Pulse Anodic Stripping Voltammetry (DPASV). Scan rate 10 mVs^{-1} with duration for 1 sec and pulse amplitude (ΔE) 25 mV. For determination of Cu(II) and Pb(II) in blood serum in patients with acute leukemia and control in the same cell.

5 mL of each sample solution and 1 mL of 0.1 M HNO_3 solution as supporting electrolyte were transferred into the electrolysis cell and completed to 10 mL using bidistilled water (pH ~ 2). The solution was deaereated by passing pure nitrogen for 16 min. The deposition potential were controlled at -1.2, -0.25, -0.6 and -0.75 V vs. Ag/AgCl sat'd KCl respectively and applied to a fresh mercury drop while the solution was stirred. After the deposition step and further 15 sec. (equilibrium time) the voltammogram was recorded.

Different concentration from the standard metal ion (individually) were added to the

cell using an automatic pipette, while keeping the deposition time constant. The solution was stirred and purged with nitrogen for 30 sec. after each spike. The concentration of each Cu(II) and Pb(II) in the electrolytic cell was calculated in the sample solutions by using standard addition method, Then the concentration in µg/ml of each blood serum in patients with acute leukemia and control were calculated and compared.

Statistical analysis

Student-t test was used to determine the difference between two techniques. A "p" value <0.05 was considered statistically significant. Statistical analyses were carried out using the SPSS® statistical software package (SPSS for Windows version 13.0, SPSS Inc., Chicago, Illinois, USA). All results are expressed as mean and standard deviation (mean ± SD).

Results and Discussion

This study included 50 patients were newly diagnosed as acute leukemia(AML: 30; ALL: 20), 20 females and 30 males aged between 20 and 50 years. Their clinical and laboratory data shown in table (1).

Optimal conditions for the determination of Cu(II) and Pb(II) by DPA_dSV technique

Preliminary experiments have been carried out to investigate the effect of various operational parameters on the differential pulse anodic stripping response. The anodic peak currents were studied with respect to the supporting electrolyte compositions, pH, deposition potential, scan rate and deposition time to optimize the conditions for analytical utility to obtain the highest peak signal for metal ions Cu(II) and Pb(II) in serum solution samples. It was noticed that, 0.01 M nitric acid solution (pH ~ 2) gave promising results for the determination of Cu (II) and Pb(II) ions.

The effect of deposition potential of each metal ion was studied and it was observed that the highest and best shape peaks for Cu(II) and Pb(II) were at deposition potentials -0.25 and -0.6 V vs. Ag/AgCl sat'd KCl. respectively. The effective scan rate which gives a suitable peak height of each metal was 10 mv/sec.

The effect of deposition time on the oxidation peak signals of these metal ions was examined. **Fig. 1** show the differential pulse anodic stripping voltammograms of Cu(II) in blood serum of patients with acute leukemia in acid solution at different deposition times. The optimal deposition times were selected for these metal ions of all sample solutions in a manner that linear relation must be established between deposition times and current signals.

Concentration of Cu(II) and Pb(II) in blood serum of patients with acute leukemia and control as determined by stripping voltammetry technique and inductively coupled plasma optical emission spectrometry.

The results of Cu(II) and Pb(II) concentrations in blood serum of control and patients with acute leukemia as determined by DPA_dSV and ICP-OES are presented in Table 2 The mean Cu(II) and Pb(II) concentrations in blood serum of patients with acute leukemia are 0.8180 and 0,2336 µg/ml, respectively, while in control are 0.4070 and 0.2988 µg/ml , respectively The results of the studied Cu(II) and Pb(II) in blood serum of patients with acute leukemia and control are discussed as follows.

Determination of Cu(II)

Fig. 1 shows the differential pulse anodic stripping voltammograms of Cu(II) in blood serum sample of patients with acute leukemia spiked with different concentration of copper ions in nitric acid solution of pH ~2. On plotting of i_p vs. Cu(II) concentrations for all blood serum samples in the same supporting electrolyte at the same conditions, straight lines are obtained (standard addition method). From the interceptions of these lines with the concentration axis at zero current signals, one can calculate the concentration of Cu(II) in each sample.

The mean serum copper level in patients with acute leukemia (0.818 µg/ml) was significantly higher than that found in control (0.407 µg/ml) as shown in Table 2; Fig. 6, with P value=0.003 (Table 3). Our result is in agreement with that of Zuo et al., 2006 (12), Demir et al., 2011 (14) and Akkuş et al., 1998 (15) in which the serum Cu level in patient with leukemia was significantly higher than that in controls. They found that the serum copper levels in patients with leukemia were 1.291 and 1.226 µg/ml and in controls were 0.867 and1.047 µg/ml respectively.

Our results are in agreement with Tessmer et al., 1972; (12) Carpentieri et al., 1986 (16) , Osman et al., 1983;(17) and Akkus et al., 1998 (15) studies in leukemic patients in which serum Cu levels have been found to be higher than those of controls. Also our results in agreement with Tessmer et al., 1972 (12) and Ilicin, 1971 (18) who suggested that serum copper would be a useful indicator for the extent of leukemia and malignant lymphoma, and might be a predictor for chemotherapy response and they added that remission is usually associated with the return of Cu levels to normal ranges.

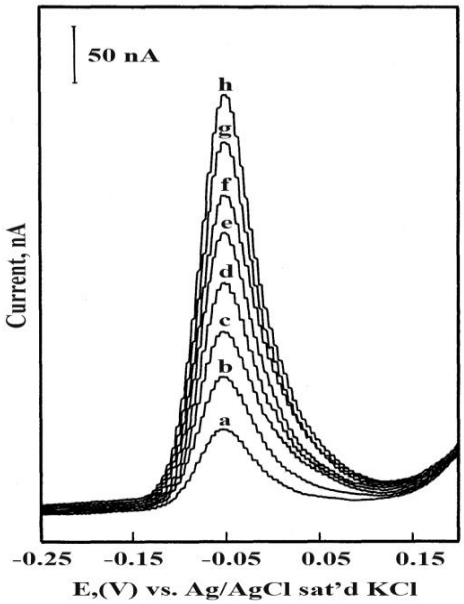

Fig. 1: DPAS Voltammograms of Cu(II) in blood serum sample of leukemia patient spiked with different concentrations of Cu(II) ions in 0.01M HNO_3 acid solution, pH ~ 2 at deposition potential -0.25V and deposition time 30 sec, (a) sample, S; (b) S+1.0×10^{-7} ;(c) S+2.0×10^{-7}; (d) S+3.0×10^{-7} ;(e)S+4.0×10^{-7} ;(f) S+5.0×10^{-7}; (g) S+6.0×10^{-7}; (h) S+$7.0\text{x}10^{-7}$ M Cu(II)**.**

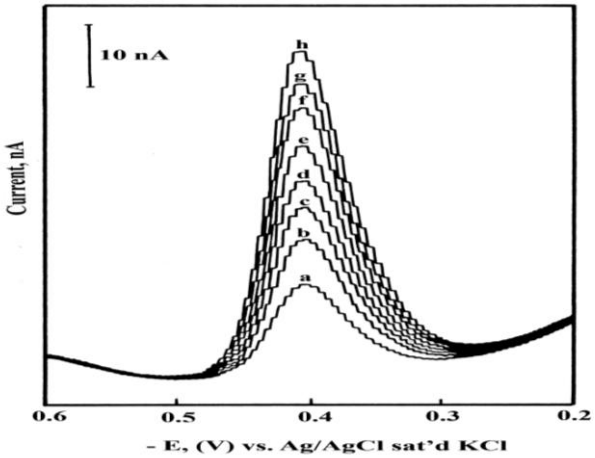

Fig. 2: DPAS Voltammograms of Pb(II) in blood serum sample of leukemia patient spiked with different concentrations of Pb(II) ions in 0.01M HNO_3 acid solution, pH ~ 2 at deposition potential -0.6V and deposition time 30 sec, (a) sample, S; (b) S+1.0×10^{-8} ;(c) S+2.0×10^{-8}; (d) S+3.0×10^{-8}; (e)S+4.0×10^{-8}; (f) S+5.0×10^{-8}; (g) S+6.0×10^{-8}; (h) S+$7.0\text{x}10^{-8}$ M Pb(II)**.**

Determination of Pb(II)

The differential pulse anodic stripping voltammograms of lead in blood serum sample solution of patients with acute leukemia in absence and in presence of standard lead ions is shown in Fig. 2. The plots of peak current against concentration, a straight lines are obtained. From the interception of these lines with the concentration axis at zero current signal gives the concentration of Pb(II) in the voltammetric cell for each sample. The mean serum lead level was lower in leukemic patients (0.2336 µg/ml) than in the control (0.2988 µg/ml) but without significant value as shown in Table 2, with P value= 0.381 (Table 3). Our results not in agreement with Demir et al, 2011 study (14) who found that levels of Pb were high in serum of acute leukemia compared to the control group but they add that literature data about Pb are more controversial and that no study evaluating Pb metal levels in acute leukemia in the literature. Further studies are necessary to interpret this finding better and to clarify the role of lead in the pathogenesis of leukemia.

Comparison of the analytical methods

Statistical analyses were carried out using the SPSS statistical software package, A "p" value <0.05 was considered statistically significant.

A comparative study was carried out between the results of Cu(II) and Pb(II) in healthy blood serums obtained using differential pulse adsorptive stripping voltammetry(DPA_dSV) and inductively coupled plasma optical emission spectrometry (ICP-OES). A comparison of the results is shown in (Table 2).

Table.1 Clinical (symptoms and signs) and laboratory data of studied patients

Clinical presentations		Laboratory data	
Symptoms and signs	**Number and percentage of total (50 patients)**	**Parameters**	**Mean ± SD**
Fever		**WBC Count ($\times10^9$/L)**	47.3 ± 23.7
Intermediate grade	7 (35 %)	**Absolute neutrophil**	
High grade	13 (65 %)	**Count ($\times10^9$/L)**	1.4 ± 0.5
Pallor	18 (90%)	**Hemoglobin level (g/dl)**	7.56 ± 2.83
Jaundice	2 (10%)	AST(IU/L)	36.7±7.8
		ALT(IU/L)	39.1±9.7
Bleeding tendency		**Platelet Count ($\times10^9$/L)**	65.8 ±34.6
Epistaxis	3 (15%)		
Per gums	4 (20%)		
Per rectum	1 (5%)		
Purpura	12 (60%)		
Vaginal bleeding	1 (5%)		
Hepato-splenomegally	8 (40%)	Creatinine (mg/dl)	0.93±0.23
Lymphadenopathy	9 (45%)		

WBC: White blood cell; AST: Aspartate transaminase; ALT: Alanine transaminase

Table.2 Serum Cu and Pb Levels of the Leukemia patients and Healthy donor samples

		Cu level(mean±SD) µg/ml		Pb level(mean±SD) µg/ml	
		DPA_dSV	ICP-OES	DPA_dSV	ICP-OES
Leukemia patients	Max	1.8372±0.212	1.9521± 0.192	0.9351±0.016	1.0350±0.016
	Min	0.1894±0.013	0.2143±0.014	0.0369±0.006	0.0380±0.002
	Mean	0.8180±0.011	0.8029±0.021	0.2336±0.013	0.2437±0.015
Healthy donor	Max	0.8516±0.045	0.9136±0. 018	0.7775±0.015	0.8174±0.016
	Min	0.1755±0.011	0.1674±0.013	0.1222±0.011	0.1338±0.016
	Mean	0.4070±0.018	0.425±0.014	0.2988±0.014	0.3087±0.015

Table.3 Serum Pb and Cu Levels of the Participants using DPAdSV

Parameter	Patient cases (n=50)	Control cases (n=30)	P
Pb (µg//ml)	0.2336±0.216	0.2988±0.213	P=0.381
Cu (µg/ml)	0.8180±0.468	0.4067±0.200	P=0.003

Pb, Lead; ; Cu, Copper

All results are expressed as mean and standard deviation (mean ± SD); P value < 0.05 was considered statistically significant.

Table.4 Comparative mean of Serum Pb and Cu Levels of the controls using ICP & DPAdSV

Parameter	Control cases (n=30)		P
	ICP	**DPAdSV**	
Pb (µg/ml)	0.399±0.213	0.299±0.213	P=0.208
Cu (µg/ml)	0.519±0.199	0.4067±0.199	P=0.136

It was proved that the results obtained using stripping voltammetry for Cu(II) and Pb(II) (0.407 and 0.2988 µg/ml, respectively) nearly in agreement with those obtained using inductively coupled plasma optical emission spectrometry of the same elements (0.519 and 0.349 \ µg/ml, respectively). For healthy control. Generally, the data obtained by inductively coupled plasma optical emission spectrometry are in close agreement with those obtained by stripping voltammetry for some metals and slight differences for the others.However,the slight differences that may be found sometimes between both techniques are mainly due to the manipulation of the analyst and metal interferences in cases of inductively coupled plasma optical emission spectrometry while the standard addition method is used to perform the stripping voltammetry technique. The standard addition method is more accurate than the calibration curves, since additions of the standard analyst to the sample give precise results and minimize or even avoid the interferences usually inherent with the matrix analysis (19).

Conclusion

In this study we found the levels of copper to be lowered and of lead to be elevated in patients with acute leukemia. Further studies are needed to clarify the role of these elements in pathogenesis of acute leukemia. And also a comparative study was carried out between the results using DPAdSV and ICP-OES techniques, which are in very good agreement (Table 4).

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