RESEARCH ARTICLE

Effects of Acute Lymphoblastic Leukemia on Ceruloplasmin Oxidase, Copper and Several Markers of Oxidative Damage, in Children

Wesen Adel Mehdi[1], Faridah Yusof[2], Atheer Awad Mehde[2,3]*, Jwan Abdulmohsin Zainulabdeen[4], Raha Ahmed Raus[2], Alaa Shawqi Abdulbari[1]

Abstract

    <u>Background</u>: **Acute leukaemia is characterized by fast growth of abnormal clones of haemopoietic precursor cells inside bone marrow leading to undue accumulation in the bone marrow. Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer. <u>Materials and Methods</u>: The study concerned 50 children diagnosed with ALL (mean age, 8.55±2.54) compared to 40 healthy controls (mean age, 8.00±1.85). The Hb, serum copper , ceruloplasmin oxidase, advanced oxidation protein products (AOPPs), total antioxidant activity (TAA) and protein were measured in all groups.One proteinous component was isolated by gel filtration chromatography from the precipitate produced by polyethylene glycol. <u>Results</u>: Significantly higher levels of AOPP, copper and decrease in total antioxidant activity were noted in the cases. Statistical analysis also showed a significant increase (p<0.01) in the activity of serum ceruloplasmin oxidase in patients with ALL compared to normal subjects .The maximum velocity (Vmax) and Michaelis constant had values of 104.2 U/L and 11.7 mM, respectively. The ΔH* values for ceruloplasmin oxidase in ALL patients were positive, confirming the reaction to be endothermic. <u>Conclusions</u>: The results from this study showed a significant increase in AOPP, ceruloplasmine oxidase and decrease in total antioxidant activity .These parameters may play a role in development of DNA damage in childhood patients with acute lymphoblastic leukemia (ALL).The ΔS* and ΔG* values were negative, these refer that the reaction of ES formation is spontaneous, but needs energy in a so-called endergonic reaction. Also the negative ΔS* value of ceruloplasmin oxidase indicates that the complex [ES*] is further modulated through increasing structure arrangement.**

Keywords: Acute lymphoblastic leukemia (ALL) - ceruloplasmin - AOPP - copper

Introduction

    Leukemia is the most common malignancy in childhood (Tharnprisan, 2013). Acute leukaemia is a malignant disorder of white cells caused by a failure of normal differentiation of haemopoietic stem cells and progenitors into mature cells (Hassanzadehet al., 2011). Leukemia results from a mutation in a single stem cell, the progeny of which form a clone of leukaemic cells. Often there is a series of genetic alteration rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of cancer-suppressing genes. The cell in which the leukaemic transformation occurs may be a lymphoid precursor, a myeloid precursor or a pluripotent stem cell capable of differentiating into both myeloid and lymphoid

cells (Bain, 2003). Acute leukaemia is a condition produced by an abnormal expression of genes, which is generally a result of chromosomal translocation (Shaikh, 2014).The disease may be created from lymphoid cells of altered lineages giving rise to B or T cell Leukemia or occasionally varied -lineage leukemia (Gaynon, 2005). Oxidative stress is known to be a noticeable feature of many acute and chronic disorder, and also cancer and leukemia (Galli et al., 2005). On other hand, Jiang et al suppose that the MTHFR C677T genotype is associated with risk of ALL in Caucasians (Jiang et al., 2013). Protection mechanisms of the body play an central role in the form of anti-oxidants and consequently, minimize the damage, familiarizing itself to the stressful conditions. Antioxidants are compounds that array, scavenge, and suppress the creation of ROS, or oppose their actions

[1]Department of Chemistry, College of Sciences for Women, University of Baghdad, [3]Department of Acceptable Analysis, Health and Medical Technical College, Baghdad, Iraq, [2]Department of Biotechnology Engineering, College of Engineering, International Islamic University Malaysia, Kuala Lumpur, Malaysia *For correspondence: atheerawod74@gmail.com

and play a main part in the deterrence of various diseases as well as cancer and their clinical appearances (Dalle et al., 2006; Uzun et al., 2007). Advanced oxidation protein products (AOPPs) are a new marker of oxidative injury, valued for their ease of determination and stability (Mera et al., 2005). AOPPs result from the action of chlorinated compounds on proteins, leading to the change of dityrosine residues and consequently to the protein cross-linking, aggregation and precipitation (Krzystek-Korpacka et al ., 2008).

Ceruloplasmin oxidase is an α2 glycoprotein (Hellman and Gitlin, 2002). This protein is a member of the multi copper oxidase family (Panichi et al., 2004). Ceruloplasmin is synthesized in the liver containing 6 atoms of copper in its structure (Hellman et al., 2002). Ceruloplasmin carries more than 95% of the total copper in human plasma. The rest is accounted for by macroglobulins (O'Brien and Bruce, 2009). Other study showed the study on patients with lung cancer, they concluded that increased oxidative stress leads to the formation of more nitrogen radicals which results in nitration and oxidation of nitrated proteins such as ceruloplasmin and oxidation of other plasma proteins (Pignatelli et al., 2001). Davis and Johnsonn , showed on their study on rats that low dietary intake of copper leads to decrease in serum ceruloplasmin oxidase level, with increasing the susceptibility of these animals to colon cancer (Davis and Johnsonn, 2002). The aim of the study is was to evaluate the correlation between ceruloplasmin oxidase and some marker of oxidative damage in the blood of ALL patients. Also study isolation, characterization and purification ceruloplasmin oxidase from sera of child with acute lymphoblastic leukemia using different biochemical techniques.

Materials and Methods

The present study was collected from fifty children with acute lymphoblastic leukemia and forty healthy children to be used as control. These patients were hospitalized at the Protection of Children Hospital Medical City in Baghdad, Iraq. Five milliliter of blood sample were collected and the blood was allowed to clot for at least 10-15 min. at room temperature, centrifuged for (10) min. at 3000 rpm. Total antioxidant activity (TAA) in serum samples was carried out according to Rice -Evans and Miller (Evans and Mille, 1994). The serum AOPP was measured by Enzyme Linked Immunosorbent Assay (ELISA) (CUSABIO BIOTECH COM.). The enzymatic assay of ceruloplasmin oxidase activity was accomplished using the modified Rice method and p-phenylene diamine-2HCL as a substrate (Erel, 1998). Serum total protein was estimated by Lowery method (Lowry et al., 1951).

Partial purification of ceruloplasmin oxidase by gel filtration chromatography

The method given had been yielded an enzyme preparation acceptable for human infusion (Hao and Wickerhauser, 1977). All steps were done at 4°C unless stated otherwise.

Polyethylene glycol 4000 (PEG) Fractionation: Solid PEG was added in the amount of 0.2 gm/10ml of

serum (Hao and Wickerhauser, 1977). All operations were conducted at 4°C. After stirring for 60 minutes, the suspension was centrifuged at 4000 rpm for 30 minutes. The supernatant contained ceruloplasmin oxidase and most of the smaller proteins (Noyer et al., 1980). The protein in precipitate and supernatant are estimate by Lowery method (Lowry et al., 1951), the ceruloplasmin oxidase had been measured in each fraction .

Dialysis: The dialysis sac containing the suspension in (Step 1) was dialyzed in contradiction of 0.015M (pH 6.9), phosphate buffer, which contained 0.1M sodium chloride. The solution had been stirred by a magnetic stirrer overnight at 4°C. The buffer was changed twice during dialysis (Robyt and White, 1987). Then and there the protein in the supernatant solution containing the enzyme was estimated by Lowery method (Lowry et al., 1951), the ceruloplasmin oxidase had been measured then stored for the next step.

Gel Filtration Chromatography: Gel filtration chromatography was used to separate serum ceruloplasmin oxidase different forms following Robyt and White method (Robyt and White, 1987), the column has a dimension of 3 × 98 cm which contained a Sephadex G-200 gel to height of 87 cm. Dependent on the volume of this column which is 450 ml, the gel was packed in water. The slurry was carefully decanted down on a glass rod to prevent air bubbles formation. A concentrated serum 4.5 ml was prepared in (Step 2), had been put to top of the bed of Sephadex G-200, followed by deionized water. Elution was carried out at a flow rate 24 ml / hour with a certain time of 10 min, by deionized water, as eluent. The protein in each fraction collected were detected by measuring the absorbance at wave length 280 nm .Peak was collective separately from the plot of an absorbance against elution volumes and ceruloplasmin oxidase was determined in each fraction.

Ceruloplasmin Oxidase-polyacrylamide gel electrophoresis

Polyacrylamide gel 7.5% was prepared by mixing 7.5 ml of distilled water, 33 ml of stock buffer (Tris-glycine 0.15 M) pH 8.9 and 22.2 ml of acrylamide solution. The mixture was degassed for 15 minutes, then 3.2 ml of ammonium per sulfate solution and 0.1 ml of N,N,N,N , tetramethylenediamine (TEMED) were added to the mixture solution (Amersham Biosciences ,1999). The mixture was gently mixed and loaded in the gel plates. The gel was allowed to polymerize for about 40 minutes. Pre electrophoresis was carried out at 50 mA and 15 v/cm for 30 min, then of 10 μl of the samples were applied into the wells in the gel. electrophoresis was continued at 40 mA and 15 v/cm for 3 hours or until the bromophenol blue dye reached the gel margin. Finally the gel was removed and have been stained ceruloplasmin oxidase activity.

Kinetic Parameters (Km and V max)

Effect of the Temperature: Ceruloplasmin oxidase enzymatic reaction was carried out in optimum reaction condition using different temperatures [25, 30, 35, 40 ,45,50,55]. The optimum temperature was evaluated by plotting the correlation between the enzyme activities

versus the temperature values.

Effect of the pH: The enzymatic reaction was carried out via buffers with different pH [4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8] for ceruloplasmin Oxidase. The pH optimum was estimated by scheming the relationship between the enzyme activities versus the pH values.

Effect of substrate concentration: Ceruloplasmin Oxidase enzymatic reaction was carried out in optimum reaction condition using altered concentrations of p-phenylene diamine-2HCL as a substrate [4.0 ,4.5, 5, 5.5, 6.0, 6.5, 7.0 mM], The correlation between each substrate concentration and the enzyme activity was plotted in order to determine the optimum substrate concentration for each enzyme activity. Then the values of Km and Vmax for ceruloplasmin toward substrate were determined by using the Lineweaver-Burk plot [the relationship between 1/V versus 1/[S]].

Thermodynamic Parameters: The thermodynamic parameters of the transition state (Ea, ΔH^*, ΔG^*, ΔS^*) were determined. All statistical analyses in studies were performed using SPSS version 15.0 for Windows [Statistical Package for Social Science, Inc., Chicago, IL, USA]. Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by student T-Test. The probability $p<0.05$=significant, $p>0.05$=non-significant.

Results and Discussion

A total of 50 of child with ALL were included in the current study. It was found that the levels of Hb was significantly decreased ($p<0.01$) as shown in Table 1 in diagnosed leukemic patients compared to control group. Anemia is a common result in patients with acute leukemia or lymphoma. The anemia is produced by a variation of mechanisms, as well as neoplastic cell infiltration into bone marrow ,also by nutritional deficiencies, and defects in erythropoietin as a result of the disease itself (Choi and Pai, 2003; Suriya and Aleem, 2012; Mehde et al., 2014).

Serum proteins are valuable signs for initial showing of any abnormal function, inflammation and diseased condition. Also proteins can differ depending on the age of the person (Sheikh et al., 2012). The current results indicate a significant decrease ($p<0.01$) in serum protein and this is consistent with other studies of (Halton et al., 1998; Khan et al., 2006; Mehdi and Abdulbari, 2013). Acute protein loss may be due to reduced protein intake coupled with a hypermetabolic state resulting in quick reduction of visceral proteins (Khan et al., 2006). The current study showed that mean copper and ceruloplasmin oxidase, have been significantly increased ($p<0.01$) compared to control group. This observation corroborates with another study in acute lymphocytic leukemia (Sgarbieri et al., 2006). Variations in serum copper concentrations had been found in lymphoproliferative conditions and also in ovarian, gastrointestinal tumors and breast (Rosas et al., 1995; Arinola and Charles-Davies M, 2008). The increase in ceruloplasmin oxidase activity in patients group was contract with another study (Gadjeva et al., 2005). More than 95% of plasma copper is bound to ceruloplasmin and this part is an indication of role of ceruloplasmin in copper transportation. The copper atom of ceruloplasmin is necessary for copper operation in the biosynthesis of cytochrome C oxidase. ceruloplasmin play such as a growth factor, regulated function of the protein; it is mediated by the enzymatic ability of ceruloplasmin to convert Fe (II) to Fe (III).Other activity of ceruloplasmin is at the border between a regulatory and an enzymatic function. Increased levels of ceruloplasmin are found in patients who have degenerative diseases, leukemia, and other malignant tumors (Harris et al., 1997).

Advanced oxidation protein products (AOPP) showed a significant increase ($p<0.001$)in patients group in comparison to control group. Advanced oxidation protein products are one of the biochemical parameters indicative of oxidation stress. The AOPP are proteins, predominantly albumin and its aggregates damaged by oxidative stress (Kalousová et al., 2005). Advanced oxidation protein products result from the action of chlorinated compounds
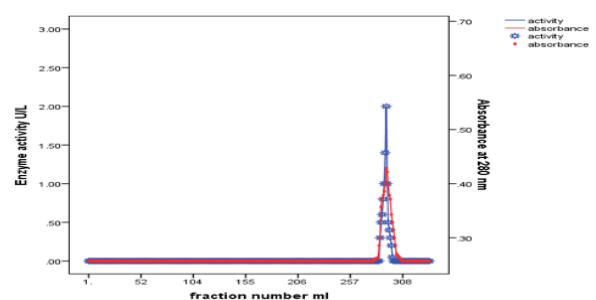
Table 1. The Mean and Standard Deviation of Hb, Serum Copper, Ceruloplasmin Oxidase, AOPP , TAA and Protein in Patients Group and Control

Characteristic	Patients group [n=50]	Control group [n=40]	p value
Hb(g/dl)	9.03±0.93	11.51±0.93	<0.01
Copper (μg/dl)	146.25±10.47	124.22±10.36	<0.01
Ceruloplasmine Oxidase(U/L)	93.63±7.42	68.23±5.92	<0.01
T.S. Protein[g/dl]	6.37±1.13	7.43±0.45	<0.01
AOPP [ng/dl]	98.23±43.66	57.47±16.59	<0.001
S. TAA [mmol/l]	0.97±0.16	1.80±0.37	<0.001

Figure 1. Gel Filtration Profile of Ceruloplasmin from ALL Patients

Table 2. Partial Purification Steps of Ceruloplasmin from ALL Patients

Purification Stage	Volume [ml]	Activity U/L	Total Activity [mU]	Total protein (g/l)	S.A U/min./mg protein	Yield	Folds of Purification
Crude	10	96.45	0.965	64.98	1.48	100	1
PEG supernatant	8.6	90.67	0.779	49.78	1.82	80.8	1.23
Dialysis	12	67.55	0.811	30.55	2.21	84	1.49
Sephadex G- 200	70	9	0.63	0.63	14.29	65.42	9.66

on proteins, leading to the formation of di-tyrosine residues and consequently to the protein crosslinking, aggregation and precipitation. Except for being the effect of oxidative imbalance, AOPPs are involved in the further development of oxidative stress and inflammation by the activation of immune cells (Kalousová et al., 2005). In this study show increase level of AOPP in patients group when compared to control that's support the notion that free radical reactions may be increased in malignant cells.A relationship between leukemia and oxidative stress has been observed. Leukemic cells produce higher amounts of ROS than nonleukemic cells because the former are experiencing sustained oxidative blockade (A l-Gayyar et al., 2007). The decrease in TAA that's indicate a lack of balance between elevated ROS generation and antioxidant capacity, which is inefficient. This situation can lead to deteriorated function of different organs (Krawczuk-Rybak et al., 2012)

Table 2 showed the purification by PEG make it particularly appropriate for the preparation of a protein that is vulnerable to proteolytic degradation such as ceruloplasmin. The PEG retain anti-proteases and may inhibit ceruloplasmin degradation (Noyer et al., 1980). The specific activity was a little increased after dialysis. This may possibly be due to the exclusion of the small molecules and increase the purification of enzyme In Table 2and Figure 1 showed that there was mainly one peak by gel filtration separations. The specific activity of the enzyme was increased in (14.29) folds than the activity in initial extract as shown in Table 2, with total activity 630 U/ml.

Figures 2 showed the electrophoresis pattern profile of crude sera and partial purified ceruloplasmin. by PEG ,Partial purified by dialysis and partial purified by Sephadex G-200. The ceruloplasmin was detected on the
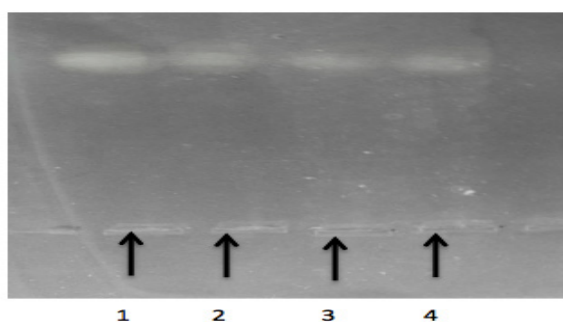

Figure 2. PAGE 7.5% Profile of Crude Serum and Partial Purified Ceruloplasmin. 1) Crude serum patients; 2) Partial purified by PEG; 3) Partial purified by Dialysis; 4) Partial purified by Sephadex G-200
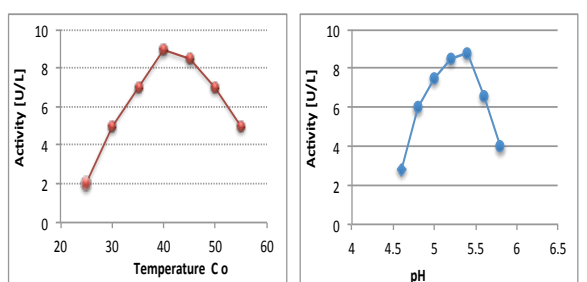

Figure 3. Effect of Temperatures and pH on Ceruloplasmin Activity

gel by exploiting its oxidase activity with PPD-2HCL as a substrate. Results in figure 1 showed that enzyme activities appeared as a single band.

Optimum conditions for ceruloplasmin activity

Optimum pH: The pH (4.6, 4.8, 5, 5.2, 5.4, 5.6, 5.8) effect have been studied on ceruloplasmin activity as shown in figure 3. The result showed that maximum enzyme activity in was at pH 5.4 in partial purified ceruloplasmin. The decrease in ceruloplasmin activity at low pH may be due to effect of pH environment of reaction in ionic groups in active site or varying in ionic state for substrate or complex enzyme-substrate at the concentration of substrate above than Km, if the substrate concentration is slight, it will depend on enzyme (William, 1974).

Effect of Temperature: In partial purified ceruloplasmin activity increases according to the incubation temperature until it reaches maximum at 35 Co as shown in Figure 3 .The role of enzyme catalyzed reactions, similar to chemical reaction, increases with temperature. This means that the initial reaction rate will rise with temperature until it becomes impossible to measure due to almost immediate inactivation. These results were nearly resembles to the other studies in normal human serum (Rahman, 1966). In practice, most enzymes are completely inactivated above (70°C) (Plummer, 1978).

Effect of substrate concentration: Determination of partial purified ceruloplasmin activity with different substrate concentration [4.0, 4.5, 5, 5.5, 6.0, 6.5, 7.0 mM], p-phenylene diamine-2HCL. Figures 4 showed
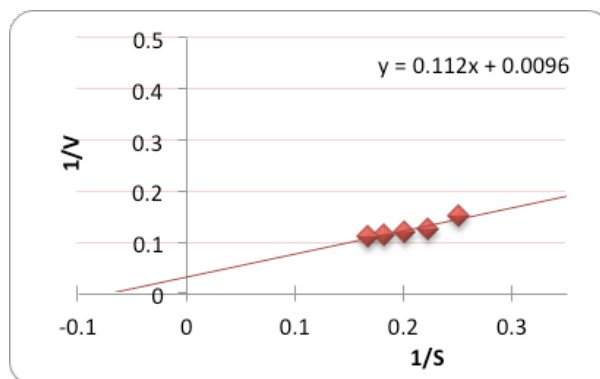

Figure 4. Determination of Km and Vmax for Ceruloplasmin Oxidase of in Partial Purified Ceruloplasmin using Lineweaver-Burk Plot
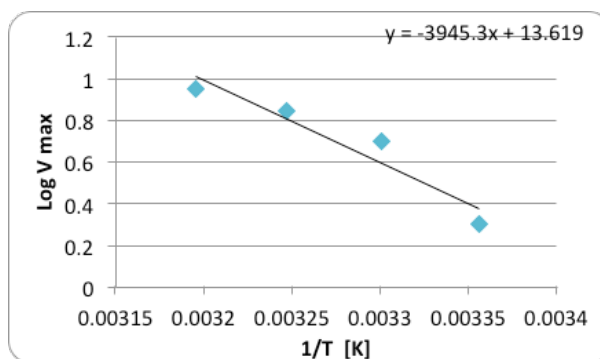

Figure 5. Arrhenius Plot between Invers Temperature and Log Vmax

Table 3. The Activation Energy and Temperature Coefficient for Ceruloplasmin Oxidase

	Ea [cal/mol]	Q10
Ceruloplasmin Oxidase	7838.72	1.04

Table 4. Thermodynamic Parameters at Transition State in Patients with Bladder cancer and normal Subjects

Case	Ea* (KJ/mol)	ΔH* (KJ/mol)	ΔG* (KJ/mol)	ΔS* (J/mol.K)
Patents with ALL	32.80	30.22	108.82	-253.48

Lineweaver– Burk plot by plotting the reciprocal of the initial velocity versus the reciprocal of the substrate concentration. A linear relationship was obtained Figure 4 giving a Vmax [104.17 U/L] and Km value of [11.67Mm]. As our knowledge no previous studies have purified and determine kinetic study of ceruloplasmin in ALL patients .

The thermodynamic parameters of the transition state were estimated from Arrhenius plot of ln K_{+1} values against (1/T) values as shown in Figure 5, which gives a linear relationship according to the following equation:

ln k= -Ea/RT+ A

The activation energy of the binding reaction was calculated from the slope of the straight line.

Where:

A: Arrhenius constant

Ea: The activation energy

R: The gas constant

T: Absolute temperature

The value of Q10 by using the equation, table 3 :

Ea= 2.3 RT2T1 log Q10/10

Q10: Temperature coefficient

R: The gas constant

T: Temperature.

Ea: The activation energy

The enthalpy of transition state (ΔH*) was determined from the following equation:

ΔH* = Ea – RT

The transition state of free energy change (ΔG*) was calculated from the following equation:

ΔG*=- R T ln K +R T ln KT/

Where:

k: is Boltzmann constant (1.38 x 10-23J.K-1).

h: is Plank constant (6.62 x 10-34J.sec).

The change in entropy of the transition state (ΔS*) was calculated from the following formula:

ΔS*= (ΔH* -ΔG*)/T

Table 4 clear the values of ΔH*,ΔS* and ΔG* in transition state for [ES] complex reaction. ΔH* value of ceruloplasmin oxidase in ALL patients was positive and this indicates that this enzyme reaction is endothermic and need energy for the formation of [ES*] complex .The ΔG* was a negative value , this indicates that the reaction of ES formation is spontaneous , but needs energy so called endergonic reaction. ΔG* value of this reaction is independent on molecular pathway of mechanism

of transformation (Devlin,1993).The ΔS* value of ceruloplasmin oxidase was negative , so indicate that the complex [ES*] more arranged than enzyme, wherever the negative value of ΔS* reverse throughout increasing the structure arrangement (Devlin,1993).

Conclusion

The results from this study showed a significant increase in ceruloplasmin oxidase, AOPP, copper and decrease in TAA, thus suggesting that these factors may be useful in following improvements in the repair of damaged cellular in subjects with ALL, However, further studies are needed to validate this measure in other populations with this treatment or with consolidation therapy[CNSphase] or in patients treated with maintenance chemotherapy.

References

Al-Gayyar M, Eissa L, Rabie A, El-Gayar A (2007). Measurements of oxidative stress status and antioxidant activity in chronic leukemia patients. *J Pharm Pharmacol*, **59**, 409-17.

Arinola O, Charles-Davies M (2008). The plasma levels of trace elements in nigerian males with different PSA Values. *Malaysian J Med Sci*, **15**, 33-6.

Bain J (2003). Leukemia Diagnosis. 3rd Edition , E-Publishing Inc., New York, PP. 53-6.

Choi J, Pai S (2003). Erythropoietic activities in acute leukemia and in malignant lymphoma with or without bone marrow involvement. *Annals of Clinical & Laboratory Science*, **33**, 407-10.

Dalle R, Rossi R, Colombo R, et al (2006). Biomarkers of oxidative stress in human disease. *Clinical Chemistry*, **52**, 601-23.

Davis C, Johnson W (2001). Dietary copper and dimethylhydrazine affect protein kinase C protein and mRNA expression and the formation of aberrant crypts in colon of rats. *Bio factors*, **15**, 11-26.

Devlin T (1993). Text Book of Biochemistry with Clinical Correlations. 3rd edition. Wiley-Liss. USA.

Erel O (1998). Automated measurement of serum peroxides activity. *Clin Chem*, **44**, 2313-19.

Evans R, Mille R (1994). Total antioxidant status in sera and body fluids. In: Methods in Enzymology, New York: Academic Press 279-93.

Gadjeva V, Kutchukova D, Aladjov E, Georgieva R (2005). Correlation between plasma malondialdehyde and ceruloplasmin activity in patients with malignant haematological diseases. *Trakia Journal of Sciences*, **3,** 29-33.

Galli F, Piroddi M, Annetti C (2005). Oxidative stress and reactive oxygen species. *Contributions to Nephrology*, **149**, 240-60.

Gaynon P (2005). Childhood acute lymphocytic leukemia and relapse. *Br J Haematol*, **131**, 579-87.

Halton J, Atkinson S, Barr R (1998). Growth and body composition in response to chemotherapy in children with

acute lymphoblastic leukemia. *Int J Cancer*, **11**, 81-4.

Hao Y, Wickerhauser M (1977). Proc. Of the International Workshop on the on the Technology for Protein Separation and Improvement of Blood Plasma Fractionation. DHEW Publication No.(NIH). pp.372-79.

Harris Z (1997). The biology of human ceruloplasin. World Scientific; Singapore pp 285-306.

Hassanzadeh J, Mohammadi R, Rajaeefard A, et al (2011). Maternal and prenatal risk factors for childhood leukemia in southern of Iran. *Iran Red Crescent Med J*, **13**, 398-403.

Hellman N, Gitlin J (2002). Ceruloplasmin metabolism and function. *Ann Rev Nutr*, **22**, 439-58.

Hellman N, Kono S, Mancini G, et al (2002). Mechanisms of copper incorporation into human ceruloplasmin. *J Biol Chem*, **277**, 46632-38.

Jiang Y, Hou J, Zhang Q, et al (2013). The MTHFR C677T Polymorphism and Risk of Acute Lymphoblastic Leukemia: an Updated Meta-analysis Based on 37 Case-control Studies. *Asian Pac J Cancer Prev*, **14**, 6357-62.

Kalousová M, Zima T, Tesar V, et al (2005). Advanced glycoxidation end products in chronic diseases-clinical chemistry andgenetic background. *Mutat Res*, **579**, 37-46.

Khan A, Sheikh M ,Intekhab K (2006). Effect of hypoproteinemia on treatment outcome in children with acute lymphoblastic leukemia. *J Ayub Medical College*, **18**, 53-6.

Krawczuk-Rybak M, Panasiuk A, Czygier M, et al (2012). Total antioxidant status (TAS) in childhood cancer survivors. Folia Histochem. *Cytobiol*, **50**, 468-72.

Krzystek-Korpacka M, PatrynE, Boehm D, et al (2008) . Advanced oxidation protein products (AOPPs) in juvenile overweight and obesity prior to and following weight reduction. *Clinical Biochemistry*, **41**, 943-49.

Lowry N, Rosebrough A, Randal R (1951). Protein measurement with the Folin phenol reagent. *J BiolChem*, **193**, 265-75.

Mehde A, Mehdi W, Zainulabdeen J, Abdulbari A (2014). Correlation of inhibin and several antioxidants in children with acute lymphoblastic leukemia. *Asian Pac J Cancer Prev*, **15**, 4843-46.

Mehdi W, Abdulbari A (2013). Study the activity of Acid , Alkaline RNase and 5-nucleotidases in sera of Child with Acute lymphoblastic Leukemia. *Australian Journal of Basic and Applied Sciences*, **7**, 72-6.

Mera K, Anraku M, Kitamura K, et al (2005). The structure and function of oxidized albumin in hemodialysis patients: its role in elevated oxidative stress via neutrophil burst. *Biochem Biophys Res Commun*, **334**, 1322-28.

Noyer M, Dwulet F, Hao Y, Putnam F(1980). Purification and characterization of undegraded human ceruloplasmin. *Anal Biochem*, **102**, 450-58.

O'Brien P, Bruce W (2009). Endogenous toxins: targets for disease treatment and prevention, 2 Volume Set. John Wiley & Sons. pp. 405-6.

Panichi V, Taccola D, Rizza G, et al (2004). Ceruloplasmin and acute phase protein levels are associated with cardiovascular disease in chronic dialysis patients. *J Nephrol*, **17**, 715-20.

Pignatelli B, Li C, Boffetta P, et al (2001). Nitrated and oxidized plasma proteins in smokers and lung cancer patients. *Cancer Res*, **61**, 778-84.

Plummer D (1978). An Introduction to Paractical Biochemistry. 2nd Edn. McGraw-Hill Book Company.UK.p. 142.

Protein electrophoresis technical manual, handbook from amersham biosciences, U.S.A. (1999).

Rahman Y (1966). Existence of a third ribonuclease in rat-liver particulates. *Biochimica et Biophysica Acta*, **119**, 470-79.

Robyt J,White B (1987). Biochemical techniques, theory and practice wadsworth, inc., belmont, california, USA.

Rosas R, Poo J, Montemayor A, et al (1995). Utilidad de la relación cobre/zinc en pacientes con linfoma o leucemias aguda o crónica. [utility of the copper/zinc ratio in patients with lymphoma or acute or chronic leukaemias]. *Rev Invest Clin*, **47**, 447-52.

Sgarbieri U, Fisberg M, Tone L, Latorre M (2006). Nutritional assessment and serum zinc and copper concentration among children with acute lymphocytic leukaemia: a longitudinal study. *Sao Paulo Med J*, **124**, 316-20.

Sheikh M, Abid R, Qureshi A, Bashee T (2012). Expression of Low MolecularWeight Proteins in Patients with Leukaemia. *West Indian Med J*, **61**, 235-39.

Sheikh M, Ali S, Khurshid M, FadooZ (2014)Chromosomal Abnormalities in Pakistani Children with Acute Lymphoblastic Leukemia. *Asian Pac J Cancer Prev*, **15**, 2907-9

Suriya O, Aleem A (2012). Frank hematuria as the presentation feature of acute leukemia. *Saudi J Kidney Dis Transpl*, **21**, 940-2

Tharnprisan P, Khiewyoo J, Sripraya P, Wiangnon S (2013). Relapse-free rate with childhood acute lymphoblastic leukemia treated under the thai national protocol. *Asian Pac J Cancer Prev*, **14**, 1127-30.

Uzun H, Konukoglu D, Gelisgen R, et al (2007). Plasma protein carbonyl and thiol stress before and after laproscopic gastric banding in morbidly obese patients. *Obesity Surgery*, **17**, 1367-73.

William J (1974). Methods in Enzymology. 3rd Edition ., Academic press. Newyork and London.