Effect of vitamin E on chemotherapy induced oxidative stress and immunoglobulin levels in patients with acute myeloid leukemia

Khalid N. Mustafa;*
*Anwar Sheikha**,*
*Imad A-J Thanoon***,*
*Farhad NA Hussein** .*
** Dept. of Internal medicine, College of Medicine- Mosul University.*
*** Dept. of Internal Medicine, College of Medicine- Hawler Medical University*
**** Dept. of Pharmacology, College of Medicine- Mosul University*

Abstract

To assess the effects of acute myeloid leukaemia (as a disease), the chemotherapy and the addition of vitamin E as an antioxidant on oxidative stress parameters(total antioxidant status , serum albumin , ferritin, ceruloplasmin) and immunoglobulin levels (IgA, IgM, IgG), in patients with acute myeloid leukaemia The study was conducted in the Medical City in Baghdad, between Nov.2004 and April 2007. Fifty individuals were included in this study, twenty-five patients with acute myeloid leukaemia and 25 age and sex matched apparently healthy subjects taken as a control for the initial laboratory tests. Initially for both the patients and control groups the following parameters were assayed, total antioxidant status , serum albumin , ferritin , ceruloplasmin and immunoglobulin levels (IgA, IgM, IgG).For the patient group after receiving chemotherapy, the same parameters were assayed, later after a 30 day course of vitamin E (400 IU/day) as an antioxidant, the parameters were also measured. Initially, there was a highly significant differences ($P<0.001$) in the serum levels of ferritin, in patients with acute myeloid leukaemia before cytotoxic therapy and in comparison with control's result . After having the specific cytotoxic regimen , there was a significant reduction in total antioxidant status , serum albumin , ferritin ,ceruloplasmin and immunoglobulins (IgA, IgM, IgG), in comparison to pre-chemotherapy results. After a course of vitamin E, there was a significant raise in serum total antioxidant status, serum albumin, ceruloplasmin and immunoglobulin with a significant reduction in serum ferritin, in comparison to postchemotherapy results.

Keywords: Acute myeloid leukemia, chemotherapy, vitamin E, oxidative stress, immunoglobulin levels.

Introduction

The cause of leukaemia is not known and free radicals have been implicated in the pathogenesis of leukaemia (1). Under normal conditions, antioxidant mechanisms scavenge reactive oxygen species (ROS) and protect the organism from the damaging effects of oxidative stress e.g those which occur with the administration of certain drugs, cellular antioxidant mechanisms may be unable to prevent the adverse impact of ROS on critical cellular processes (2).

In the living system and aerobic organism, a complex antioxidant mechanisms has been evolved to stand against the uncontrolled free radicals (3). These antioxidant acts together in consent to form an integrated antioxidant system(4), which could be classified into, primary antioxidants as albumin, ceruloplasmin (5) , secondary antioxidants as superoxide dismutase (as an enzymatic antioxidant) and ascorbate (as a non-enzymatic entity) (6,7), and the tertiary antioxidants as proteases (3). The aggressive chemotherapy is the cornerstone of cancer therapy and some reports suggest that endogenous antioxidants are reduced in patients with cancer , while other showed that the administration of antineoplastic agents during cancer chemotherapy results in much greater degree of oxidative stress than is induced by cancer itself (8).

The aim of this study is to assess the oxidative status (through measurement of total antioxidants status, serum albumin , ferritin and cerulopasmin) and immunoglobulin levels (IgA, IgG, IgM) in

patients with acute myeloid leukaemia (AML)after a course of cytotoxic therapy and the effect of adding vitamin E (as an antioxidant) on these parameters under study.

Patients and Methods

The study was conducted in the Medical City in Baghdad, between November 2004 and April 2006. Cases were selected according to certain criteria, which include proved cases of acute myeloid leukaemia according to clinical , hematological and bone marrow results, planned to be on a specific cytotoxic regimen , and cooperative patients.

Out of 36 patients with acute myeloid leukaemia, only 28 were included in this study and only 25 completed the study. They were 19 males and 6 females with a mean age of 52.12±7.63 year . Also included in this study 25 apparently healthy persons, 20 males and 5 females with a mean age of 54.52±5.98 year as a control.

A 10 ml venous blood samples were taken from both patients (before starting planned cytotoxic regimen , by the end of a course of the planned cytotoxic regimen and after a course of vitamin E) and the controls at the initial step for comparison with AML patients before starting cytotoxic regimen. (Planned cytotoxic regimen : Daunorubicin 45mg/m2 IV days 1,3,5. Cytosine arabinoside 100 mg/m2 IV every 12 h from day 1-7).

Serum immunoglobulin (IgG, IgA, IgM) were measured by single radical immunodiffusion (RID) method ,total antioxidant status was measured by peroxidase /H2O2/ABTs colorimetric assay ,serum ferritin levels were measured by immunoturbidimetric immunoassay method, serum ceruloplasmin level were measured by an automated bichromatic micromethod analysis by assy of its p-phenylenediamine oxidase activity as described by Hohbadel et al., (1975) (9).Finally serum albumin concentration was measured by Bromocresol Green Method(Johnson et al,1999) (10).

Comparison of parameter results within each group paired t-test was used, to compare parameters results with the control, unpaired t-test was used .All values quoted as the mean ±SE and a P value of <0.05 were considered to be statistically significant.

Results

Initially before starting chemotherapy and in comparison to controls, patients with AML , showed a non significant differences in the parameters under assay with the exception of serum ferritin which showed a highly significant differences (P< 0.001)(Table 1).

Serum levels of TAS , albumin, ferritin, ceruloplasmin were significantly lowered (P< 0.001), after a course of specific chemotherapy (Table 2).

All the parameters under assay (TAS, serum albumin, ferritin , ceruloplasmin and immunoglobulins) showed a significant raise in their values after a one month course of vitamin E (Table 3).

By comparing levels of parameters under assay before chemotherapy and after antioxidant in patients with AML there was a significant differences in levels of albumin, ferritin and immunoglobulins , with insignificant differences in the levels of TAS and ceruloplasmin (Table 4).

Discussion

There was insignificant differences in the levels of all the parameters under assay in patients with AML in comparison to controls, with the exception of serum ferritin which showed a highly significant differences . Ferritin levels was found to be raised in patients with acute lymphocytic leukaemia , also pretreatment ferritin levels was found to be elevated in patients with myeloblastic leukaemia (11).

The increased synthesis and release of ferritin by the leukaemic blasts were blamed to be responsible for increased ferritin concentration (12). Albert et al, reported that serum ferritin levels closely followed the activity of the disease . The increased pretreatment serum ferritin levels have normalized completely when the patients achieved a complete remission (13).

This study also revealed a significant reduction in TAS after a course of chemotherapy . The sum of endogenous and food derived antioxidants represents the total antioxidant capacity of extracellular fluids which integrates the cumulative effects of all antioxidants present in the plasma and body fluids and may give a more relevant biological information as compared to that

obtained by individual parameters (14). This study deals with the measurement of both TAS and some of the individual antioxidant parameters (serum albumin, ferritin and ceruloplasmin). Very few studies have assayed the antioxidant status of patients with malignancies, Senturker et al and Malvy et al, reported that endogenous antioxidants are reduced in patients with malignant disease as compared to normal counter parts (15,16).

Bakan et al, by measuring the concentration of malondialdehyde (MDA) as an indicator of lipid peroxidation and serum glutathione with glutathione reductase (as an enzymatic secondary antioxidant system) , concluded that there is a significant changes in patients with chronic lymphocytic leukaemia (17).

In agreement with the present results Ladas et al, who suggested that total antioxidant status declines during cancer treatment (18). Kennedy et al reported that TAS decreases in children with acute lymphoblastic leukaemia during the first six months of therapy (19).

Gadjeva et al reported a raise in MDA serum levels and a reduction in superoxide dismutase (as an indicator of antioxidant activity) and concluded that after polychemotherapy , the oxidative stress and imbalance of antioxidant enzyme system significantly progresses in patients with lymphoproliferative hematological disease (20).

A part from the renal loss of low molecular weight water soluble antioxidants , an increased metabolic consumption and the degradation of antioxidants in plasma and tissues resulting from oxidative stress induced by chemotherapy , may have contributed to the decrease of plasma antioxidant concentration (21).

This study also revealed a significant reduction of serum albumin , ferritin and ceruloplasmin after a course of chemotherapy .

Multiple mechanism have been proposed to explain ceruloplasmin antioxidant activity including scavenging of superoxide and other reactive oxygen species and inhibiting the fenton reaction by conversion of Fe+2 to Fe+3 (ceruloplasmin is also called ferroxidase) (22).,In addition there is evidence that ceruloplasmin as an antioxidant block protein and DNA damage and that it affords protection against free radical initiated cell injury and lysis (23).

With regard to immunoglobulin levels (IgA, IgG, IgM) , this study reported a significant reduction in immunoglobulin levels in AML patients after a course of chemotherapy.

The first report about effects of chemotherapy on immunoglobulin levels came in 1971 , by Borella and Webster (24) , who reported that there is an immunosuppressive effects of longterm combination chemotherapy in children with acute leukaemia in remission . Solanki et al reported in their study that there is a normal or low levels of immunoglobulins with different diseases including leukaemia and lymphoma (25). Nilsson et al founded that there was a loss of antibodies against measles and rubella in children treated with an intensive chemotherapy and suggested the reimmunization of those patients with lymphoblastic leakaemia as a necessary step after completion of the cytotoxic regimen (26). Luczynski et al concluded that humeral immunity impairment in children with acute lymphoblastic leukaemia is an effect of treatment not the disease (27).

This study revealed a significant raise in TAS , serum albumin , ceruloplasmin with a significant reduction in serum ferritin levels after a one month course of vitamin E as an antioxidant following the cytotoxic course.

There is question need an answer, does the administration of antioxidants during cancer chemotherapy affect the antineoplastic efficiency or the development of side effects?. Factors to be considered in the design of studies to answer the question posed above include , the properties of the individual antioxidants, the mechanism of action of the antineoplastic agents and the mechanism where by antineoplastic agents cause their side effects . Additionally the impact of chemotherapy induced oxidative stress upon the efficacy of antineoplastic agents and the role of ROS may play in drug –induced apoptosis need to be elucidated (28).

Extensive in vitro studies and limited in vivo studies have revealed that individual antioxidants such as vitamin A, vitamin E, vitamin C and carotenoids , induce cell

differentiation and growth inhibition to various degrees in rodent and human cancer cells by complex mechanisms . The proposed mechanisms for these effects include inhibition of protein kinase C activity , prostaglandin E1-stimulated adenylate cyclase activity . Furthermore , antioxidant vitamins individually or in combination enhance the growth inhibitory effects of irradiation, chemotherapeutic agents, hyperthermia and biological response modifiers on tumor cells (29). Also a study conducted by Lamson and Brignall concluded that there is no reduction of efficacy of chemotherapy or radiation , when given with antioxidant and on the contrary they suggest increased effectiveness of many cancer therapeutic agents as well as decrease in adverse effects (28).

With regard effects of vitamin E administrations on immunoglobulin levels (IgA,IgG,IgM) after a course of cytotoxic regimen , this study demonstrated a significant raise in the levels of immunoglobulins after a course of vitamin E. Vitamin E was found to play a role in blocking the formation of cancer promoting nitrosamine and helping to enhance immune function (30).

It might be regarded as the first study that involved follow up of patients with AML from the initial diagnosis before starting specific cytotoxic regimen , after a course of specific cytotoxic regimen and after a course of vitamin E as an antioxidant, evaluating effects of cytotoxic regimen and antioxidant therapy on oxidative status (by measuring TAS, serum albumin, ferritin and ceruloplasmin) and immunoglobulin levels (IgA, IgG, IgM).

As a conclusion, chemotherapy in patients with AML , do affect oxidative status as reflected by the reduction in TAS, serum albumin, ferritin and ceruloplasmin and immunoglobulin levels (IgA, IgG, IgM), and giving vitamin E as an antioxidant do improve TAS, serum albumin and ceruloplasmin as well as immunoglobulin levels. Further studies to evaluate the addition of an antioxidant to the cytotoxic regimen in patients with leukaemia might be needed, taking in consideration pharmacology and pharmacokinetic parameters of both antioxidant and cytotoxic agents with the staging of leukaemia , clinical and laboratory outcome of each case.

References

1. Singh V; Ghalaut PS; Kharb S et al. Plasma concentration of lipid peroxidation products in children with acute leukaemia . Indian J Med Sc 2001 Apr; 55(4) :215-217.

2. Conklin KA. Cancer chemotherapy and antioxidants. J Nutri 2004;134:3201S-3204S.

3. Murray RK. Metabolism of xenobiotic and red and white blood cells. In : Harpers Biochemistry , 25th ed , Murray RK, Granner DK, Mayes PA, Rodwell VW editors . McGraw-Hill, Appleton and Lange . PP.781-786.

4. Jacob RA . The integrated antioxidant system . Nutri Res 1995; 15:755-766.

5. Stahl W and Sies H. Antioxidant defense. Vitamin E, C and carotinoids . Diabetes 1997;46:514-518.

6. Wohaieb SA; Godin PV. Starvation and related alteration in free radical tissue defense mechanism in rats . Diabetes 1987;36:169-173.

7. Rose RC; Bode AM . Biology of free radical scanvenger : An evaluation of ascorbate . FASEBJ 1993; 7(12) :1135-1142.

8. Conklin KA. Dietary antioxidants during cancer chemotherapy : Impact on chemotherapeutic effectiveness and development of side effects . Nutri Cancer 2000;37:1-18.

9. Hohbadel Dc;McNeely MD;Sunderman Fw.Automated bichromatic analysis of serum ceruloplasmin.Ann Clin Lab Sci 1975 Jan-Feb;5(1):65-70.

10. Johnson AM; Rohlfs EM; Silverman LM. Proteins . In Tietz textbook of clinical chemistry . 3rd ed. , Buritis CA and Ashwood ER editors . 1999 W.B Saunders company . USA : PP 477-540.

11. Hann HL; Levy HM; Evans AE . Serum ferritin as a guide to therapy in neuroblastoma . Cancer Res 1980; 40:1411-1413.

12. Thomas CE ; Morehouse LA; Aust SD. Ferritin and superoxide-dependant lipid peroxidation. J Biol Chemist 1985; 260(6) :3275-3280.

13. Albert E; Fromm H ; Hornemann . Ferritin in acute leukaemia . serum ferritin

concentration a non- specific tumor marker for M1 and M2 myeloid leukaemia . Med Klin (Munich) 1991 Jun ; 86(6) : 297-304.

14. PapaGeorgiou M; Stiakaki E; Dimitriou H et al . Cancer chemotherapy reduces plasma total antioxidant capacity in children with malignancies . Leukaemia Res 2005;29:11-16.

15. Senturker S; Karahalil B; Inal M et al . Oxidative DNA base damage and antioxidant enzyme levels in childhood acute lymphoblastic leukaemia. FEBS Lett 1997; 416:286-290.

16. Malvy DJ; Arnand J; Burtschy B et al . Antioxidant micronutrients and childhood malignancy during oncological treatment. Med Paediat Oncol 1997; 29:213-217.

17. Bakan N; Tarsi S ; Yilmaz O et al . Glutathione peroxidase, glutathione reductase , Cu-Zn superoxide dismutase activities , glutathione , nitric oxide and malondialdehyde concentrations in serum of patients with chronic lymphocytic leukaemia . Clin chem. Act 2003 Dec; 338(1-2) :1439.

18. Ladas EJ; Jacobson JS; Kennedy DD, et al . Antioxidants and cancer therapy : A systematic review. J Clin Oncol 2004 ; 22(3) :517-528.

19. Kennedy DD;Ladas EJ;Rheingold RS et al. Antioxidant status decreases in children with acute lymphoblastic leukaemia during the first six month of chemotherapy.Paediat Blood Cancer 2004;44(4):378-385.

20. Gadjeva V; Kuchukova D; Georgieva R. Influence of polychemotherapy on the antioxidant levels and lipid peroxidation in patients with lymphoproliferative diseases. Comparative Clin Path 2005 Aug ; 14(1) :13-18.

21. Weijl NI; Holman GD ; Wipkink-Bakkera et al. Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients . Annals of Oncol 1998; 9:1331-1337.

22. Samokyszyn VM ; Miller DM; Reif DW et al. Inhibition of superoxide and ferritin –dependent lipid peroxidation by ceruloplasmin . J Biol Chemist 1989;264(1) :21-26.

23. Ehrenwald E ; Chisdm GM; Fox PL . Intact human ceruloplasmin oxidative modifies low density lipoprotein . J Clin Invest 1994; 93:1493-1501.

24. Borella L; Webster RG. The immunosuppressive effects of longterm combination chemotherapy in children with acute leukaemia in remission . Cancer Res 1971 Apr; 31(4) :420-426.

25. Solanki RL; Anand VK; Arora HL. Serum immunoglobulin in leukaemia and malignant lymphoma. J Indian Med Assoc 1990 Nov. ; 88(11):305-307.

26. Nilsson A; DeMilito A; Engstrom P et al . Current chemotherapy protocols for childhood acute lymphoblastic leukaemia induces loss of humoral immunity to viral vaccination antigen . Paediat 2002; 109(6):1-6.

27. Luczynski W; Stasiak-Barmuta A; Krawczuk-Ryhak M et al. Is cellular immunity not impaired after remission induction in acute lymphoblastic leukaemia in children ? Pol Merkuriusz Leuk 2004;16(91):17-21.

28. Lamson DW ; Brignall MS . Antioxidants in cancer therapy ; their actions and interactions with oncology therapies . Altern Med Rev 1999; 4(5) :304-329.

29. Prasad KN; Kumar A; Kochupillai V et al . High doses of multiple antioxidant vitamin . Essential ingredients in improving the efficacy of standard cancer therapy . J Am Coll Nutr 1999; 18(1) :13-25.

30. Sung L ;Greenberg ML; Koren G et al. Vitamin E: The evidence for multiple roles in cancer . Nutri Cancer 2003;46(1):1-14.

Table (1) : Comparison between prechemotherapy AML patients and controls with regards to TAS,Albumin,Ferritin ,Ceruloplasmin and Immunoglobulins

Parameters	AML patients prechemotherapy	Control	P-value
	Mean± S.E	Mean± S.E	
TAS (mmol/l)	1.43±0.052	1.46±0.07	N.S
Albumin (g/dl)	3.57±0.25	3.5±0.17	N.S
Ferritin (µg/l)	319.33±7.6	76.4±17.7	0.0014***
Ceruloplasmin(mg/dl)	29.00±4.9	31.0±7.36	N.S
IgA(mg/dl)	240±10.09	227.00±8.6	N.S
IgG(mg/dl)	864.20±30.06	828.4±29	N.S
IgM(mg/dl)	181.16±5.6	170.6±5.6	N.S

N.S: Non significant; ***: Highly significant differences

Table (2): Comparison between pre and postchemotherapy in AML patients with regard TAS,Albumin,Ferritin, Ceruloplasmin and Immunoglobulins.

Parameters	Pre-chemotherapy	Post-chemotherapy	P-value
	Mean± S.E	Mean± S.E	
TAS (mmol/l)	1.43±0.052	1.412±0.049	0.001***
Albumin (g/dl)	3.57±0.25	2.91±0.13	0.001***
Ferritin (µg/l)	319.33±7.6	134.66±4.48	0.001***
Ceruloplasmin(mg/dl)	29.00±4.9	19.60±5.76	0.001***
IgA(mg/dl)	240±10.09	172.00±7.27	0.001***
IgG(mg/dl)	864.20±30.06	604.60±25.33	0.001***
IgM(mg/dl)	181.16±5.6	129.00±4.79	0.001***

*** Highly significant differences

Table (3): Comparison between postchemotherapy and postantioxidant in AML patients with regard TAS,Albumin,Ferritin,Ceruloplasmin and Immunoglobulins.

Parameters	Post-chemotherapy	Post-antioxidant	P-value
	Mean± S.E	Mean± S.E	
TAS (mmol/l)	1.412±0.049	1.433±0.050	0.001***
Albumin (g/dl)	2.91±0.13	3.37±0.16	0.001***
Ferritin (µg/l)	134.66±4.48	122.66± 2..96	0.031*
Ceruloplasmin(mg/dl)	19.60±5.76	27.40±5.02	0.001***
IgA(mg/dl)	172±7.27	195.40±7.04	0.005***
IgG(mg/dl)	604.60±25.33	711.20±28.9	0.001***
IgM(mg/dl)	129.00±4.79	151.40±5.19	0.001***

* significant difference ;***: highly significant differences

Table (4): Comparison between prechemotherapy and postantioxidant in AML patients, with regard TAS, Albumin,Ferritin, Ceruloplasmin and Immunoglobulins.

Parameters	Pre-chemotherapy	Post-antioxidant	P-value
	Mean± S.E	Mean± S.E	
TAS (mmol/l)	1.432±0.052	1.433±0.050	N.S
Albumin (g/dl)	3.57±0.25	3.37±0.16	0.005***
Ferritin (µg/l)	319.33±4.48	122.66± 2..96	0.001***
Ceruloplasmin(mg/dl)	29.00±4.9	27.40±5.02	N.S
IgA(mg/dl)	240.60±10.09	195.40±7.04	0.001***
IgG(mg/dl)	864.20±30.06	711.20±28.9	0.001***
IgM(mg/dl)	181.16±5.6	151.40±5.19	0.001***

N.S: Non significant difference; ***:highly significant differences