VITAMIN E AND N-ACETYLCYSTEINE AS ANTIOXIDANT ADJUVANT THERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

BY

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ABSTRACT

For the past years, cancer therapies have experienced tremendous setbacks because of an associated toxic response and free radicals formation resulting in significant numbers of treatment-induced deaths rather than disease-induced fatalities. Awareness regarding historic numbers of unsuccessful outcomes has forced patients to look for alternatives to bolster survival odds and physicians to study antioxidant use as adjunctive treatment in cancer. So, this study aimed to evaluate the antioxidant role of N-acetyl cysteine (NAC) and vitamin E in overcoming treatment- induced toxicity in cancer. Forty children newly diagnosed with acute lymphoblastic leukemia (ALL); Twenty children (group I) have taken NAC and vitamin E supplementations with conventional treatment protocol and the other twenty children (group II) have not taken any adjuvant antioxidants therapy.They were evaluated by laboratory variables (blood levels of glutathione peroxidase (Glu.Px) antioxidant enzyme, malondialdehyde (MDA) substance, tumor necrosis factor α (TNF-α), liver enzymes, bone marrow picture and clinically by incidence of complications. Results revealed reduced chemotherapy and radiotherapy toxicity as evidenced by decreasing level of MDA, TNF-α, increasing level of Glu. Px and decrease of hematological complications, toxic hepatitis, need for blood and platelets transfusions, and periods of febrile neutropenia in patients who had taken antioxidants than group II. NAC and vitamin E have been shown to be effective as antioxidant adjuvant therapy in children with ALL.

INTRODUCTION

Cancer survivors are increasing nowadays; many of them are highly motivated to seek information about dietary supplement use and complementary nutritional therapies to improve their response to treatment (Chelf et al., 2001). There is a debate about the concurrent use of anti-oxidants with cytotoxic therapies (Moss, 2006). It is true that much remain unknown concerning antioxidants, their mode of action and possible interaction.

Vitamin E succinate (VES, alpha tocopherol succinate) as antioxidant has generated some interest as an adjunctive cancer therapy due to its remarkable lack

of toxicity in vivo (Bendich and Machlin, 1988). If given with omega 3 fatty acids of fish oil, it may prolong survival in patients with generalized malignancy (Gogos et al., 1998).

Another antioxidant is N-acetylcysteine (NAC) which is the acetylated form precursor of L-cysteine and reduced glutathione. It was used as a mucolytic agent in respiratory illness as well as an antidote for acetaminophen hepatotoxicity but recently used as complementary therapy of cancer (Arora- Kuruganti et al., 1999; Chiao et al, 2000). Children undergoing treatment for acute lymphoblastic leukaemia (ALL), receive multi-agent chemotherapy, many of them (cytosine arabinoside, doxorubicin, cyclophosphamide and methotrexate) are associated with free radical production so may affect the antioxidant status during therapy (Lamson and Brignall, 1999).

This study aims to evaluate the role of N-acetylcysteine and alpha tocopherol succinate as adjuvant therapy in children with ALL during the induction and CNS intensification phases of chemotherapy.

SUBJECTS AND METHODS

This study was conducted on 40 children with acute lymphoblastic leukaemia (18 males and 22 females) with their ages ranged between 2-15 years. They were recruited consecutively from those admitted to Pediatric Hematology and Oncology Unit at Mansoura University Children's Hospital, Mansoura, Egypt, in the period between November 2006 and March 2007.

Diagnosis of acute lymphoblastic leukaemia was done according to standard methods including morphological, cytochemical and immunological evaluation.

The patients were treated by the modified BFM 76/79 protocol of therapy (Henze et al.,1981) which is the most widely used protocol for the treatment of childhood ALL. They were divided into 2 groups. The first group (group I) included 20 patients (10 males and 10 females) who were supplemented with vitamin E (Alpha tocopherol succinate) in a dose of 400 IU/day orally and N-acetylcysteine (NAC) in a dose of 600 mg/day orally (Richardson et al., 2000) in addition to chemotherapy from day one of diagnosis till the end of CNS intensification (Phase II of chemotherapy). The second group (group II) included the other 20 patients (8 males and 12 females) who received chemotherapy alone without any supplementation. Informed consent was obtained from the parents prior to giving chemotherapy and any supplementation.

Blood samples were collected from

every patient at day 28 of induction and at the end of CNS intensification for estimation of Malondialdehyde (MDA) thiobarbituric acid reactive substance, serum glutathione perioxidase, tumor necrosis factor alpha (TNF- α) and liver enzymes. Blood was collected into heparin tubes which were processed as soon as possible after sampling. Bone marrow picture was done for both groups. Leishman stained film (Merck/BDH/Merck House, UK) was done for bone marrow aspiration.

The patients were evaluated by incidence of complications like haematological complications (bone marrow hypoplasia, febrile neutropenia), need for blood and platelets transfusions and toxic hepatitis which was diagnosed by elevation of liver enzymes after chemotherapy, exclusion of viral hepatitis and liver enzymes were normalized when chemotherapy were temporary suspended (Hadir et al., 2001).

Determination of Malondialdehyde (MDA) "Thiobarbituric acid reactive substances" (Draper et al., 1993):

Serum proteins are precipitated by addition of trichloroacetic acid (TCA). Then, thiobarbituric acid (TAB) reacts with malondialdehyde (MDA) to form thiobarbituric acid reactive product, which is measured colorimetrically at 534nm.

Estimation of serum glutathione peroxidase (Paglia and Valentine, 1967):

Glutathione peroxidase was assayed by kit purchased form Randox (Randox Laboratories Ltd., UK) (Cat. No. RS 505). This method based on the fact that; glutathione peroxidase (Glu. Px) catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. Then decrease in absorbance at 340 nm is measured calorimetrically.

Determination of human tumor necrosis factor alpha (TNF-α) by ELISA technique (Biosource, Belgium) according to method of Beutler and Cerami (1987).

Estimation of liver enzymes; serum glutamate oxaloacetic transaminase (SGOT) and glutamate pyruvic transaminase(SGPT) according to method of Reitman and Frankel(1957).

Statistical analysis:
The Statistical analysis of data was done by using Excel program and SPSS program (Statistical Package for Social Science Version 10). The description of data was done in the form of mean + SD for quantitative data and frequency and proportion for qualitative data. The analysis of data was done to test statistical

significant difference between groups. For qualitative data, Chi-square test was used. For quantitative data, student t-test was used to compare between two groups, P is significant if < 0.05.

RESULTS

Considering serum Glu.Px, table (1) shows a significant increase in its level in group I who received antioxidants. There is a statistically significant difference ($p<0.05$) between both groups after CNS intensification.

As regard of MDA and serum TNF-a (table, 1), their levels were decreasing in group I more than the group II. However, there was no significant statistical difference.

Regarding occurrence of hematological complications (bone marrow hypoplasia and times of occurrence of febrile neutropenia) between the two groups, the incidence is more in group II with a highly statistically significant difference ($p<0.001$) as shown in table (2).

As regards toxic hepatitis, there is more incidence of toxic hepatitis in group II after induction with a significant statistical difference between the two groups ($p<0.05$) and after phase II of chemotherapy with a highly significant statistical difference ($p<0.001$) (Table, 3).

Table (4) shows that the liver enzymes (SGOT and SGPT) are more elevated in the group II than group I after induction and phase II of chemotherapy with a significant statistical difference between the two groups ($p<0.001$, <0.05, <0.001 and <0.001 respectively).

Looking to number of units of blood transfusion and platelets bags, there is generally more incidence of transfusion in group II than group I with a highly significant statistical difference between the two groups after induction and phase II of therapy ($p<0.001$) (Table, 5).

DISCUSSION

Many patients with cancer take antioxidant nutritional supplements during cancer treatment to alleviate treatment toxicities and to improve long-term outcomes, but little is known about the efficacy and safety of antioxidants use during cancer treatment (Ladas et al., 2004).

Despite considerable debate about the role of antioxidant status in cancer outcomes, very few studies have assessed changes in antioxidant status and oxidative stress. Among children undergoing treatment for cancer there were only one study to our knowledge measure exposure and treatment outcome in pediatric oncology (Kennedy et al., 2005)

who found that children with ALL have altered antioxidant status at diagnosis and during treatment. Results of Kennedy et al., supports prior observations that children with ALL have altered antioxidant and micronutrient status at diagnosis and during treatment.

In the present study, the antioxidant status was associated with the increased treatment related oxidative stress (prolonged duration of neutropenia, increased usage of growth factor and elevated liver enzymes) in group II (children with All who didn't receive antioxidants). These findings are consistent with the results of Ray et al., (2000), Portakal et al. (2000) and Mantovani et al., (2002) who found an increase of oxidative stress during treatment in cancer patients as compared with controls.

Also that is confirmed in the present study by looking to MDA level among group I and group II : giving NAC and vitamin E had decreased the level of free radicals resulting from oxidation as evidenced by lowering level of MDA and increasing the level of Glu. Px in group I who take supplementation. Overall, a significant difference was observed as regard serum Glu. Px between group I and group II after CNS intensification but not on day 28 of induction.

Comparing intensity of chemotherapy

taken during induction phase and CNS intensification it will be found nearly similar as during induction vincristine + Doxorubicin are used mainly but during CNS intensification cyclophosphamide plus cytosine arabinoside and oral 6 mercaptopurine are used in addition to prophylactic cranial irradiation which is proven to elicit ten times more free radicals than chemotherapy alone. Looking to level of TNF-α, there was obvious decreasing level (with no statistical significance) after phase II of CNS intensification with intake of vitamin E and NAC.

Considering the high levels of Glu.Px and low levels of MDA after CNS intensification among cases received supplementations, it can be understood the increased incidence of toxic hepatitis among group II compared to group I who experienced less incidence of toxic hepatitis, shorten duration of febrile neutropenia which is the period needed to stay in hospital and use of highly cost growth factor. These results support the findings of Mantovani et al., (2003, 2004) who conclude the effective role of some antioxidants in reducing reactive oxygen species, proinflammatory cytokines levels and increasing Glu. Px levels in cancer patients at different sites. In this study, the effective in vivo use of NAC as antioxidant to counteract chemotherapy and radiotherapy toxicity in cancer patients in concomitant with vitamin E confirm the beneficial effect of its in vitro

use in advanced stage cancer by Mantovani et al.,(2000).

As regards blood and platelet transfusion, addition of antioxidants supplementation had significantly decreased the need of blood and platelet transfusion during induction phase and CNS intensification.

Results from this study supported our hypotheses that oxidative stress increase during standard chemotherapy and radiotherapy of cancer patients. With the combined use of NAC and vitamin E, some improvement in oxidative status (MDA and serum Glu.Px) and TNF-α had occurred and this is an encouraging result. It is to be taken into account that this adjuvant therapy is relatively low-cost drugs; therefore, it may be considered as having

a favorable cost- benefit profile, whereas achieving an optimal patient compliance. Findings in this study suggest that these two antioxidants may be protective against treatment related oxidative stress and toxicity in children with ALL.

Before a recommendation for NAC and vitamin E supplementation can be made, future studies with a larger sample size are needed to confirm this observation.

Table (1): Serum glutathione peroxidase, malondialdehyde and tumor necrosis factor-α in different phases of therapy of both leukemia groups.

Groups	Glutathione peroxidase (U/L)		MDA(nmol/ml)		TNF-α (pg/ml)	
	After phase I	After phase II	After phase I	After phase II	After phase I	After phase II
Group I mean±SD	901.48±57.87	1158.74±265.03	3.47±0.85	2.81±1.19	42.48±29.90	40.90±60.05
Group II mean±SD	946.49±112.13	947.94±122.08	3.52±0.87	3.44±1.14	38.28±32.52	37.58±24.10
P value	0.12	0.002*	0.79	0.09	0.67	0.81

MDA: Malondialdehyde.   TNF-α: Tumor necrosis factor-α.

*P is significant if ≤0.05.

Table (2): Comparison of hematological complications (Bone marrow hypoplasia and febrile neutropenia) in both leukemia groups.

Complications	Group I		Group II		Chi-square
	n.	%	n.	%	P
Negative	12	60	0	0	
Positive	8	40	20	100	<0.001***
Total	20	100	20	100	

***P is highly significant if <0.001.

Table (3): Toxic hepatitis in both leukemia groups at different phases of chemotherapy.

Toxic hepatitis	Group I		Group II		Chi-square
	n.	%	n.	%	P
After phase I chemotherapy					
Negative	14	70.0	8	40.0	0.02*
Positive	6	30.0	12	60.0	
After phase II chemotherapy					
Negative	12	60.0	0	-	<0.001***
Positive	8	40.0	20	100.0	

*P is significant if ≤0.05.   ***P is highly significant if <0.001.

Table (4): Liver enzymes levels of leukemia patients after phase I and II of chemotherapy.

Liver enzymes (U/ml)	Group I mean±SD	Group II mean±SD	t-test P
SGOT after phase I of therapy	72.79±74.26	163.0±60.13	<0.001***
SGPT after phase I of therapy	131.80±70.7	248.30±156.74	0.004*
SGOT after phase II of therapy	183.55±124.53	485.70±280.01	<0.001***
SGPT after phase II of therapy	175.55±126.51	715.90±235.14	<0.001***

*P is significant if ≤0.05.   ***P is highly significant if <0.001.

Table (5): Blood and platelets transfusion during phase I and phase II of therapy.

Blood and platelet transfusion	Group I mean±SD	Group II mean±SD	t-test P
Phase I of therapy:			
Blood transfusion	1.44±0.52	2.60±0.69	<0.001***
Platelets transfusion	1.20±0.44	3.40±1.50	<0.001***
Phase II of therapy:			
Blood transfusion	1.16±0.40	2.20±0.42	<0.001***
Platelets transfusion	1.00±0.00	1.60±0.69	<0.001***

***P is highly significant if <0.001.

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فيتامين هـ و الأستيل سيستايين كمضادات أكسدة علاجية مساعدة فى الأطفال المصابين بسرطان الدم الحاد

المشتركون فى البحث

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إرتبط علاج السرطان فى السنوات السابقة باستجابة سمية وتكوين شقائق حرة طليقة تؤدى إلى تزايد عدد الوفيات الناتجة عن العلاج وليس المرض، وقد دفع هذا الإدراك كثير من المرضى للبحث عن بدائل تزيد من فرص البقاء، ولذلك تزايدت دراسة إستخدام مضادات الأكسدة كعلاج مساعد.

تهدف هذه الدراسة إلى تقييم دور عقار فيتامين هـ والأستيل سيستايين كمضادات أكسدة فى التغلب على الآثار السمية للعلاج التقليدى للسرطان، تمت دراسة أربعين طفلاً مصابين بسرطان الدم الحاد، عشرين طفلاً قد تم إعطائهم فيتامين هـ والأستيل سيستايين كعلاج مساعد للعلاج التقليدى المستخدم وباقى الأطفال لم يتعاطوا أية مضادات أكسدة.

وتم تقييم المرضى فى الفترة من نوفمبر ٢٠٠٦ إلى مارس ٢٠٠٧ ببعض المتغيرات المعملية مثل مقياس مستوى الجلوتاثيون بيروكسيداز (Glu.Px) والمالونداى ألدهايد (MDA)، وعامل موت الورم (TNF)، وظائف الكبد وصورة النخاع العظمى والمتغيرات الإكلينيكية مثل مضاعفات المرضى، وأسفرت النتائج عن تحسن مستوى الجلوتاثيون بيروكسيداز (Glu.Px)، وانخفاض فى مستوى المالونداى ألدهايد (MDA)، وعامل موت الورم (TNF)، وقلت نسبة حدوث المضاعفات مثل الالتهاب الكبدى السمى، والحاجة لنقل الدم والصفائح الدموية وفترات إرتفاع درجات الحرارة الناتجة عن نقص كريات الدم البيضاء، وذلك فى المرضى الذين تم إعطائهم مضادات الأكسدة أكثر من المجموعة الضابطة.

وقد أظهر فيتامين هـ، والأستيل سيستايين فعاليتهما فى تقليل الأثر السمى للعلاج الكيميائى والإشعاعى فى مجموعة الأطفال المصابين بمرضى سرطان الدم الحاد.