Nutritional and anti-oxidant state in plasma of leukemic patients

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Abstract

Deficiency of many vitamins and minerals has been associated with depressed immunity. Good epidemiological evidence on the relationship between nutrition and cancer were reviewed.

The present study was undertaken to assess the nutritional state of patients with different types of leukemia as indicated by plasma levels of the vitamins A (and its precursor beta-carotene), E & C, some trace elements (Zn, Cu & Se), albumin & total thiol.

For this purpose blood specimens were taken from 105 newly diagnosed leukemic patients. They consisted of 27 patients with acute lymphocytic leukemia (ALL), 27 with acute myelogenous leukemia (AML), 25 with chronic lymphocytic leukemia (CLL) & 28 with chronic myelogenous leukemia (CML). Only 86 of those patients were followed up, they were 28 with ALL, 21 with AML, 26 with CLL & 21 with CML.

The results were compared with those obtained from 50 healthy individuals matching in age and sex.

The leukemic patients showed a significant reduction in plasma beta-carotene, vitamins A, E & C, albumin, total thiol, Zn & Se levels. While plasma Cu & Cu / Zn ratios were significantly elevated. All above-mentioned parameters were normal or near to normal on admission.

Introduction

Nutrition is a life sustaining process by which elements of nature are assimilated and used for growth and development for maintenance of healthy tissues and as mediators of physiological and metabolic processes.

Deficiency of most vitamins and minerals have been associated with depressed immunity and epidemiological evidence on the relationship between nutrition and cancer were reviewed in which diet appeared to play an important role in carcinogenesis. However, apart from an inverse association with intake of fruits and vegetables the role of specific foods and nutrients remains largely undefined.

The effectiveness of antioxidant defense system is dependent on adequate dietary intake of food containing antioxidants such as vitamins (E and C) and the metal cofactors required for antioxidant enzymes. So the antioxidant status of the body can be considerably influenced by diet and should the normal defense mechanisms be weakened by nutritional deficiencies followed by the appearance of pathogenetic consequences.

The aim of the present study was to clarify the relationship of some vitamins (A and its precursor beta-carotene, E & C), and some trace elements Zn, Cu and Se, and their related to the level of some antioxidants (albumin & total thiol) in some of leukemic patients before starting the course of treatment and after remission.

Subjects and Methods:

A) Subjects:

One hundred and five newly diagnosed leukemic patients, with age range of 14–75 years and 50 healthy normal individuals of matched age and sex were involved in the study.

The leukemic group comprised 27 patients with ALL, 27 with AML, 25 with CLL & 21 with CML, with male to female ratio of 2:1.

They were admitted to the Center of Blood Disease and Medical City Hospital from August 2001 to September 2002.

Leukemia diagnosis was based on clinical history, physical examination, blood & bone marrow study. All patients were treated by cytoxic chemotherapy.

B) Blood Specimens:

A total of 10 ml venous blood was aspirated from each patient and control subject in EDTA containing tubes. Plasma was separated by centrifugation and stored at -30°C pending analysis.

C) Methods:

Measurement of plasma beta-carotene was done by extraction with chloroform, petroleum ether and chloroform according to the method described by Puske & Kaplan (1987). Vitamin A was measured by trimethyl acetic acid reagent after extraction with chloroform, petroleum ether and acetic hydrochloride mixture.

Vitamin E was measured according to Emmerie-Angle procedure using FeCl₃ reagent after
removal of interferences by absolute ethanol and
heptane.

Measurement of plasma vitamin C was done
by oxidation with Cu\(^{2+}\) ions and formation of colored
complexes with 2,4-dihydro-phenylhydrazine in sulfuric
acid after deproteinization of plasma sample by
metaphosphoric acid.

Plasma total thiol was measured according to
Ellman's method, and plasma albumin by bromocresol
green in acidic pH.

For trace elements (Zn & Cu) flame atomic
absorption spectrophotometer (type Shimadzu, Model
AA-676, Japan) was used after 1:10 dilution with
demineralized water, while plasma Se was measured by
flameless atomic absorption spectrophotometer (type
Shimadzu, Model AA-680G, Japan) was used directly
by dispensing 10 μL of plasma sample into the
graphic furnace using AS 1 autosampler.

Results and Discussion

Results in tables (1 - 4) show a significant
decrease in plasma beta-carotene, vitamins (A, E &
C), thiol group, zinc and selenium with a significant
increase in plasma copper and Cu : Zn ratio in all
patients which returns to near normal after complete
treatment.

Many studies indicated that oxidative stress
mediated by increased level of reactive oxygen
species (ROS) had a causative role in the
pathogenesis of several types of cancer. Potentially
noxious oxygen free radicals are generated continuously
in humans.

Under physiological conditions, the human body
has developed a complex antioxidant defense
system sufficient to protect the cells against oxidative
damage.

However oxidative stress could result from a
loss of this protective balance under abnormal
conditions by overproduction of reactive oxygen
derived free radicals or inadequate antioxidant defense
systems.

Some of the effects of lipids peroxides and
ROS include epithelial cell injury and dysfunction
alteration in membrane fluidity, altered membrane
damage to plasma and proteins, enhanced adhesion
and activation of neutrophils, platelet aggregation,
increased uptake of the low density lipoproteins
(LDL) in vessel wall, decreased protein synthesis,
homocysteine and increased production of toxic
aldehydes.

For all what mentioned above carcinoma
cancer patients may need to be provided by antioxidants
to overcome the effects of lipid peroxidation and
ROS.

The present results revealed a significant
lowering in the level of beta-carotene in plasma of all
types of leukemic patients when compared with their
controls, which returned to normal after
successful treatment and at remission state (tables 1 -
4).

Higher beta-carotene or total carotenoids
were thought to associate reduced risk of coronary
heart disease and some types of cancer
and its reduction may be attributed to its role in scavenging
superoxides in cultured cells, either by chemical
interaction or physical quenching. It is also
believed that, beta carotene could play a role in
limiting LDL oxidation, as it is transported in plasma
mainly in LDL.

The low level of vitamin A in plasma of the
leukemic patients of the present study is in accord
with other reports in other types of cancer, and
maintaining adequate vitamin A status was found to
be important since it can minimize the biocconversion of
beta-carotene to vitamin A and thereby minimize its
recovery to serum as beta-carotene. In addition,
vitamin A was reported to have a direct effect on iron
metabolism. Deficiency of this vitamin produces
anemia that can be reversed by its administration
without a change in iron intake.

Results from animal experiments demonstrated that
high vitamin A intake increases the oxidative activity
to ferric ion and enhances the biocconversion of
beta-carotene.

The decrease in vitamin E level with
increased oxidative stress as presented by low plasma
level confirms other reports. Other studies showed
an inverse association between vitamin E level and
risk of mortality from cancer.

Vitamin E appears to be at the first line of
defense against peroxidation of polyunsaturated fatty
acids contained in cell and cytoplasmic membrane
phospholipids. It protects fat in LDL from oxidation
and is effective at high concentration of oxygen.

Further the ability of alpha-tocopherol to
neutralize free radicals makes it the subject of
a number of cancer prevention studies.

The present results also showed a marked
significant reduction in plasma vitamin C in leukemic
patients collectively when compared with the controls
which also returned to normal or near normal values
at remission, a finding which agrees with previous
report that showed the presence of an inverse
association between various micronutrients, mostly
vitamin C, E and beta-carotene and the risk of cancer.

It has also been reported that high intake of
vitamin E had decreased the incidence of different
types of cancer by protecting indispensable
cellular structures in the body such as proteins,
lipids, carbohydrates and nucleic acids (DNA & RNA)
from damage by free radicals and ROS that can be
generated during normal metabolism as well as
through exposure to toxic and pollutants 27. Other mechanisms may involve detoxification of carcinogens and enhancement of immune defense 28.

Reduction in plasma thiol group in the patients of the present study agrees with many previous reports on other types of malignancies 29,30 and glutathione GSH has been suggested, by many workers, to be a critical factor in protecting organisms against toxicity and diseases with maintaining membrane integrity. Its reduction is believed to be due to the increase in its consumption as a result of increased toxic radical generation 31. The inhibitory action of glutathione on peroxidation depends on vitamin E and often seen to act in a synergistic way in this respect 32.

The elevation in plasma thiol group by cytotoxic drugs may be due to free amine acid utilization and altered synthesis of GSH in cancerous cells 33. This will eliminate a part of the oxidative stress that might be seen in these patients before submission to therapy.

Hypalbuminemia of cancerous patients could be ascribed to a defective synthesis, intravascular dilution by increased plasma volume or gastrointestinal loss 34. This low albumin was found to be associated with a decrease in total peroxyl trapping capacity of serum 35.

The increase in the catalysis of major copper-containing plasma proteins (cytochrome) or the displacement of copper from ceruloplasmin by the peroxyl radical, or the use of Cu as a cofactor for many antioxidant enzyme systems are possible causes of hyper-copperemia seen in malignancy 36.

Zinc, on the other hand, is decreased as a result of malignancy, something that could be attributed to the increased use of this metal by the cells as a protecting agent against free radicals including the superoxide ions that are produced during the disease 37.

As a result of an elevation in plasma Cu and reduction in plasma Zn results in an increase in Cu/Zn ratio, which have been considered as a diagnostic and prognostic tool for different types of cancer 38. It is well known that zinc antagonizes copper and that lowering serum zinc results in more binding sites in the albumin for non-specific transport of copper with a consequent increase in the plasma copper in cancerous patients 39.

The relationship between serum selenium (Se) and malignant disease has been previously reported 40. Selenium is a component of selenoproteins, some of which have important enzymatic functions as the glutathione peroxidase and thioredoxin reductase which were found to support the activity of vitamins E and C in limiting the oxidation of lipids 41. Reduction in plasma Se at
Table 1: Plasma variable levels in ALL patients before and after treatment (All results are presented as µmol / L, except albumin as g / L)

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>50</td>
<td>27*</td>
<td>22**</td>
</tr>
<tr>
<td>Beta-carotene</td>
<td>1.02 ± 0.33</td>
<td>0.28 ± 0.14</td>
<td>0.86 ± 0.11</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>2.4 ± 0.35</td>
<td>0.79 ± 0.30</td>
<td>1.85 ± 0.21</td>
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<tr>
<td>Vitamin E</td>
<td>0.32 ± 0.09</td>
<td>0.10 ± 0.03</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>89.79 ± 17.03</td>
<td>89.71 ± 17.05</td>
<td>63.02 ± 5.7</td>
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<tr>
<td>Total thiol</td>
<td>777.5 ± 15.2</td>
<td>418.4 ± 79.85</td>
<td>714.2 ± 72.0</td>
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<tr>
<td>Albumin</td>
<td>41.0 ± 4.5</td>
<td>27.0 ± 7.40</td>
<td>42.0 ± 4.0</td>
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<tr>
<td>Copper (Cu)</td>
<td>16.30 ± 3.20</td>
<td>26.70 ± 5.10</td>
<td>16.20 ± 1.26</td>
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<tr>
<td>Zinc (Zn)</td>
<td>13.80 ± 2.5</td>
<td>8.60 ± 0.20</td>
<td>14.80 ± 2.4</td>
</tr>
<tr>
<td>Cu / Zn</td>
<td>1.03 ± 0.28</td>
<td>3.3 ± 1.1</td>
<td>1.03 ± 0.3</td>
</tr>
<tr>
<td>Selenium (Se)</td>
<td>0.14 ± 0.04</td>
<td>0.07 ± 0.02</td>
<td>0.12 ± 0.02</td>
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</tbody>
</table>

* Significant differences between the patient group & their controls before treatment for all variables.
** Significant differences between the patients before and after treatment for all variables.
Note: *p* < 0.0005 for all plasma variable values

Table 2: Plasma variable levels in AML patients before and after treatment (All results are presented as µmol / L, except albumin as g / L)

<table>
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<tr>
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<tr>
<td></td>
<td>50</td>
<td>27*</td>
<td>21**</td>
</tr>
<tr>
<td>Beta-carotene</td>
<td>1.02 ± 0.33</td>
<td>0.25 ± 0.09</td>
<td>0.80 ± 0.09</td>
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<tr>
<td>Vitamin A</td>
<td>2.4 ± 0.35</td>
<td>0.98 ± 0.35</td>
<td>1.94 ± 0.19</td>
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<tr>
<td>Vitamin E</td>
<td>0.32 ± 0.09</td>
<td>0.09 ± 0.02</td>
<td>0.24 ± 0.02</td>
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<tr>
<td>Vitamin C</td>
<td>89.79 ± 17.03</td>
<td>26.30 ± 6.24</td>
<td>65.30 ± 6.51</td>
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<tr>
<td>Total thiol</td>
<td>777.5 ± 15.2</td>
<td>408.5 ± 130.8</td>
<td>724.5 ± 58.8</td>
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<tr>
<td>Albumin</td>
<td>44.0 ± 4.5</td>
<td>26.0 ± 6.5</td>
<td>43.0 ± 6.0</td>
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<tr>
<td>Copper (Cu)</td>
<td>16.50 ± 3.20</td>
<td>29.8 ± 0.02</td>
<td>16.17 ± 0.03</td>
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<tr>
<td>Zinc (Zn)</td>
<td>13.80 ± 2.5</td>
<td>8.4 ± 0.09</td>
<td>15.0 ± 6.0</td>
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<tr>
<td>Cu / Zn</td>
<td>1.03 ± 0.28</td>
<td>3.28 ± 1.1</td>
<td>1.0 ± 0.30</td>
</tr>
<tr>
<td>Selenium (Se)</td>
<td>0.14 ± 0.04</td>
<td>0.07 ± 0.02</td>
<td>0.13 ± 0.02</td>
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</table>

* Significant differences between the patient group & their controls before treatment for all variables.
** Significant differences between the patients before and after treatment for all variables.
Note: *p* < 0.0005 for all plasma variable values
### Table 3: Plasma variable levels in CLL patients before and after treatment (All results are presented as µmol/L, except albumin as g/L)

<table>
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<tr>
<th>Plasma variables</th>
<th>Normal controls No.</th>
<th>Before treatment No. 23*</th>
<th>After treatment No. 20**</th>
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<td>20</td>
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<tr>
<td>Beta-carotene</td>
<td>0.92 ± 0.53</td>
<td>30.0 ± 0.68</td>
<td>0.85 ± 0.07</td>
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<tr>
<td>Vitamin A</td>
<td>4.3 ± 0.35</td>
<td>25.0 ± 0.19</td>
<td>1.83 ± 0.21</td>
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<tr>
<td>Vitamin E</td>
<td>0.32 ± 0.09</td>
<td>0.09 ± 0.03</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>89.7 ± 17.03</td>
<td>77.4 ± 1.34</td>
<td>16.2 ± 2.80</td>
</tr>
<tr>
<td>Total thiol</td>
<td>77.5 ± 15.2</td>
<td>87.2 ± 2.07</td>
<td>15.3 ± 2.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>44.0 ± 5.3</td>
<td>3.57 ± 1.0</td>
<td>1.13 ± 0.2</td>
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<tr>
<td>Copper (Cu)</td>
<td>16.30 ± 3.20</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>1.80 ± 2.5</td>
<td>3.57 ± 1.0</td>
<td>1.13 ± 0.2</td>
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<tr>
<td>Cu / Zn</td>
<td>1.4 ± 0.14</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>

* Significant differences between the patient group & their controls before treatment for all variables.
** Significant differences between the patient group & their controls after treatment for all variables.

### Table 4: Plasma variable levels in CMI patients before and after treatment (All results are presented as µmol/L, except albumin as g/L)

<table>
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<th>Plasma variables</th>
<th>Normal controls No.</th>
<th>Before treatment No. 28*</th>
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</tr>
<tr>
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<tr>
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<tr>
<td>Vitamin E</td>
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<td>0.09 ± 0.03</td>
<td>0.24 ± 0.02</td>
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<tr>
<td>Vitamin C</td>
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<td>16.2 ± 2.80</td>
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<td>1.13 ± 0.2</td>
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<tr>
<td>Copper (Cu)</td>
<td>16.30 ± 3.20</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.01</td>
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<tr>
<td>Zinc (Zn)</td>
<td>1.80 ± 2.5</td>
<td>3.57 ± 1.0</td>
<td>1.13 ± 0.2</td>
</tr>
<tr>
<td>Cu / Zn</td>
<td>1.4 ± 0.14</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.01</td>
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</tbody>
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* Significant differences between the patient group & their controls before treatment for all variables.
** Significant differences between the patient group & their controls after treatment for all variables.

### References

الخلاصة

في هذه الدراسة، تم الكشف عن فوائد العناصر النادرة في الجسم من حيث النوبات السكري. وقد تشير الدراسات التي أجريت إلى وجود علاقة بين العناصر النادرة ونوبات السكري. وتشمل هذه الدراسات ما هو النتائج التي توصلت إليها. بالإضافة إلى ذلك، تشير النتائج إلى أن العناصر النادرة قد تلعب دورًا في الوقاية من نوبات السكري. وتتضمن هذه النتائج أيضًا الدراسة التي أجريت في هذا الميدان.

أي قسم من الفيزيولوجيا، العناصر النادرة في الجسم من حيث النوبات السكري، جامعًا، وتشير الدراسات التي أجريت إلى وجود علاقة بين العناصر النادرة ونوبات السكري. وتشمل هذه الدراسات ما هو النتائج التي توصلت إليها. بالإضافة إلى ذلك، تشير النتائج إلى أن العناصر النادرة قد تلعب دورًا في الوقاية من نوبات السكري. وتتضمن هذه النتائج أيضًا الدراسة التي أجريت في هذا الميدان.

27 ALL, 27 AML, 27 CL & 28 CML