Nutritional and anti-oxidant state in plasma of leukemic patients

Abeer H. Mustafa[1], Ghassan A.Al-Shamma[2]
[1] Department of Chemistry, College of Science, Al-Mustansiria University
[2] Department of Chemistry & Biochemistry, College of Medicine, Al-Nahrain University

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 myelocytic leukemia (AML), 23 with chronic lymphocytic leukemia (CLL) & 28 with chronic myelocytic leukemia (CML), only 86 of those patients were followed up, they were 22 with ALL, 21 with AML, 20 with CLL & 23 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 at remission.

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 [1].

Deficiency of most vitamins and minerals have been associated with depressed immunity [2,3] 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 [1].

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 co-factors 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 pathogenomonic consequences [4].

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 its related to the level of some antioxidants (albumin & total thiol) in sera 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, 23 with CLL & 28 with CML, with male to female ratio of 1:.

They were admitted to the center of blood disease and Medical City Hospital from August 2001 to September 2002.

Leukaemia diagnosis was based on clinical history, physical examination, blood & bone marrow study. All patients were treated by cytotoxic 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 –20° C pending analysis.

C) Methods :

Measurement of plasma beta-carotene was done by extraction with ethanol, petroleum ether and chloroform according to the method described by Peace & Kaplan (1987) [5], while vitamin A was measured by trifluoro acetic acid reagent after extraction with chloroform, petroleum ether and acetic anhydride mixture [5].

Vitamin E was measured according to Emmerie-Angle procedure using $FeCl_3$ reagent after

ISSN: 1814-5922

removal of interferences by absolute ethanol and n-heptane[3].

Measurement of plasma vitamin C was done by oxidation with Cu^{+2} ions and formation of colored complex with 2,4 dinitro-phenyl hydrazine in sulfuric acid after deproteinization of plasma sample by metaphosphoric acid [3].

Plasma total thiol was measured according to Ellmans method [8], and plasma albumin by bromocresol green in acidic pH [9].

For trace elements (Zn & Cu) flame atomic absorption spectrophotometer (type Shimadzu, Model AA-646, Japan) was used after 1:10 dilution with deionized 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 graphite furnace using AS I 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 returned to near normal after complete remission.

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 toxic 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 cell's against oxidative damage.

However oxidative stress could result from a loss of this protective balance under abnormal conditions by over-production of 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 permeability to ions and proteins, enhanced adhesion and activation of neutrophils, platelet aggregation, increased uptake of the low density lipoproteins (LDL) in vessel wall, decreased protein synthesis, inactivation of enzymes and increased production of toxic aldehydes [10].

For all what mentioned above cancerous patients may need to be provided by antioxidants so 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 back to normal after successful treatment and at remission state (tables 1 - 4).

Either beta-carotene or total carotenoids were thought to associate reduced risk of coronary heart disease and some types of cancer [11], and its reduction may be attributed to its role in scavenging superoxides in cultured cells [4], either by chemical interaction or physical quenching [12]. It is also believed that beta carotene could play a role in limiting LDL oxidation as it is transported in plasma mainly in LDL [4].

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 [13], and maintaining adequate vitamin A status was found to be important since it can minimize the bioconversion of beta-carotene to vitamin A and thereby maximizes its recovery in 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 [14].

Results from animal experiments demonstrated that high vitamin A intake decreased the dioxygenase activity and the bioconversion of beta-carotene [15].

The decrease in vitamin E level with increased oxidative stress as presented by low plasma thiol confirms other reports [16-18]. Other studies showed an inverse association between vitamin E and onset or mortality from cancer [17].

Vitamin E appears to be at the first line of defense against peroxidation of poly-unsaturated fatty acids contained in cellular and subcellular membrane phospholipids. It protects fat in LDL from oxidation and is effective at high concentration of oxygen. Further more the ability of alpha- tocopherol to neutralize free radicals made it the subject of a number of cancer prevention studies [20,21].

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 [22] that showed the presence of an inverse association between various micronutrients, mostly vitamins C, E and beta-carotene and the risk of cancer [23-25].

It has also been reported that high intake of vitamin C had decreased the incidence of different types of cancer [26] by protecting indispensable molecules in the body such as proteins, lipids, carbohydrates and nucleic acids (DNA & RNA) from damage by the free radicals and ROS that can be generated during normal metabolism as well as

through exposure to toxins 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 [29,31], and glutathion 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 free radical generation [32]. The inhibitory action of glutathion on peroxidation depends on vitamin E and both were seen to act in a synergetic way in this respect [33].

The elevation in plasma thiol group by cytotoxic drugs may be due to free amino acid utilization and altered synthesis of GSH in cancerous cells [34]. This will eliminate a part of the oxidative stress that might be seen in these patients before submission to therapy.

Hypoalbuminemia of cancerous patients could be ascribed to a defective synthesis, intravascular dilution by increased plasma volume or gastrointestinal loss [35]. This low albumin was found to be associated with a decrease in total peroxyl trapping capacity of serum [33].

The increase in the catabolism of major copper-containing plasma proteins (ceruloplasmin) or the displacement of copper from ceruloplasmin by the peroxynitrite radicals, or the use of Cu as a co-factor for many antioxidant enzyme systems are possible causes of hypercupremia seen in malignancy [37,38].

Zinc, on the other hand, is decreased as result of malignancy, something that could be attributed to the increased use of this metal by the cell as a protecting agent against free radicals including the superoxide ions that are produced during the disease [39].

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 [1]. 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 in plasma copper in cancerous patients [42].

The relationship between serum selenium (Se) and malignant disease has been previously reported [43]. Selenium is a component of selenoproteins, some of which have important enzymatic functions as the glutathion peroxidase and thioredoxin reductase which were found to support the activity of vitamins E and C in limiting the oxidation of lipids [44,45]. Reduction in plasma Se at remission may be a reflection of selenium sequestration by tumor cells as a result of poor dietary intake [46]. Several mechanisms for the cancer prevention by Se were proposed by Combs and Gray in 1998 [47].

No significant difference was found between different groups of leukemic patients.

Table 1: Plasma variable levels in ALL patients before and after treatment (All results are presented as µmol / L, except albumin as g / L)

Plasma variables	Normal controls No. 50	Before treatment No. 27*	After treatment No. 22**
Beta-carotene	1.02 ± 0.33	0.28 ± 0.14	0.86 ± 0.11
Vitamin A	2.4 ± 0.35	0.79 ± 0.30	1.85 ± 0.21
Vitamin E	0.32 ± 0.09	0.10 ± 0.03	0.24 ± 0.02
Vitamin C	89.79 ± 17.03	89.71 ± 17.03	63.02 ± 5.7
Toral thiol	777.5 ± 15.2	418.4 ± 79.85	714.2 ± 72.0
Albumin	44.0 ± 4.5	27.0 ± 7.40	42.0 ± 4.0
Copper (Cu)	16.30 ± 3.20	26.70 ± 5.10	16.20 ± 1.26
Zinc (Zn)	15.80 ± 2.5	8.60 ± 0.20	14.80 ± 2.4
Cu / Zn	1.03 ± 0.28	3.3 ± 1.1	1.01 ± 0.3
Selenium (Se)	0.14 ± 0.04	0.07 ± 0.02	0.12 ± 0.02

* 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)

Plasma variables	Normal controls No. 50	Before treatment No. 27*	After treatment No. 21**
Beta-carotene	1.02 ± 0.33	0.25 ± 0.09	0.80 ± 0.09
Vitamin A	2.4 ± 0.35	0.98 ± 0.35	1.94 ± 0.19
Vitamin E	0.32 ± 0.09	0.09 ± 0.02	0.24 ± 0.02
Vitamin C	89.79 ± 17.03	26.30 ± 6.24	65.30 ± 6.51
Toral thiol	777.5 ± 15.2	408.5 ± 123.8	724.5 ± 58.8
Albumin	44.0 ± 4.5	26.0 ± 6.5	43.0 ± 6.0
Copper (Cu)	16.30 ± 3.20	29.8 ± 0.02	16.17 ± 0.03
Zinc (Zn)	15.80 ± 2.5	8.4 ± 0.09	15.0 ± 0.03
Cu / Zn	1.03 ± 0.28	3.28 ± 1.1	1.0 ± 0.30
Selenium (Se)	0.14 ± 0.04	0.07 ± 0.02	0.13 ± 0.02

* 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)

Plasma variables	Normal controls No. 50	Before treatment No. 23*	After treatment No. 20**
Beta-carotene	1.02 0.33	0.30 ± 0.08	0.85 0.07
Vitamin A	2.4 ± 0.35	0.79 ± 0.19	1.83 = 0.21
Vitamin E	0.32 0.09	0.09 ± 0.03	0.24 = 0.02
Vitamin C	89.79 17.03	25.0 ± 4.00	64.16 = 7.9
Total thiol	777.5 ± 15.2	374.0 ± 78.5	720.1 = 45.2
Albumin	44.0 ± 4.5	26.0 ± 7.0	41.7 = 3.0
Copper (Cu)	16.30 ± 3.20	27.4 ± 3.14	16.2 = 2.0
Zinc (Zn)	15.80 2.5	8.72 ± 2.07	15.3 = 2.6
Cu / Zn	1.03 ± 0.28	3.37 ± 1.0	1.1 ± 0.2
Selenium (Se)	0.14 ± 0.04	0.07 ± 0.01	0.12 ± 0.01

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

Plasma variables	Normal controls No: 50	Before treatment No.28*	After treatment No:23**
Beta-carotene	1.02 ± 0.33	0.29 ± 0.09	0.85 ± 0.17
Vitamin A	2.4 = 0.35	0.86 ± 0.26	1.85 ± 0.19
Vitamin E	0.32 = 0.09	0.11 ± 0.02	0.23 ± 0.02
Vitamin C	89.79 ± 17.03	25.6 ± 0.06	56.8 ± 7.4
Total thiol	777.5 + 15.2	378.0 ± 67.7	730.3 ± 51.4
Albumin	44.0 ± 4.5	26.0 ± 7.0	41.6 ± 1.90
Copper (Cu)	16.30 ± 3.20	26.7 + 4.24	15.4 ± 2.20
Zinc (Zn)	15.80 ± 2.5	8.03 ± 2.07	15.3 2.60
Cu / Zn	1.03 ± 0.28	3.0 ± 0.49	1.03 = 0.20
Selenium (Se)	0.14 ± 0.04	0.63 ± 0.02	0.13 = 0.03

* 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

References

1. Alexander J. W. and Peck M. D. Future Consideration for Nutrition. In: Total Parental Nutrition. Fischer J., Little Brown Comp., Eds. Boston, 2nd Ed., 1991, p.p. 447-466.
2. Tellado J. M. and Christon N. V.: Nutrition and Immunity. In: Total Parental Nutrition. Fischer J., Little Brown and Company, Eds. Boston, 2nd Ed., 1991, p.p. 475.
3. Tietz N. W.: Textbook of Clinical Chemistry. Sanders W. B. Comp., London. 2nd Ed., 1986.
4. Duthie G. G., Wahle K. W. J., and James W. P. T.: Nutr. Res. Rev.; 2: 51-62, 1989.
5. Pesce A. J. and Kaplan L. A.: Methods in Clinical Chemistry. The C.V. Mosby Company, Washington D.C., Toronto, 1987. p.p. 590.
6. Nedd J. B. and Pearson W. N.: J. Nutr.: 79:454-460. 1963.
7. Hashim S. A. and Schaffinger G. R.: Am. J. Clin. Nutr.; 19:137-45, 1966.
8. Ellman G. L.: Arch. Biochem. Biophys.: 2:70 - 77. 1959.
9. Metz A. and Schutte A.: Clin. Chem.; 13:123, 1975.
10. Walsh S. W.: Hypertens. Pregnancy; 13:1 - 32, 1994.
11. Kritchevsky S. B.: J. Nutr.; 129:5 - 8, 1999.

12. Stahl W. and Sies H.; Diabetes; 46:(suppl.2) S14 –S18, 1997.
13. Eldin A. K. and Applequist L. A.; Lipids; 31:671 –701, 1996.
14. Garcia- Cost M. N., Leets I. and layrisse M.: J. Nutr.; 130:5 –9, 2000.
15. Hung C. ,Tung Y.,Chen C. and et.al.: J. Nutr.; 130: 534 –540, 2000.
16. Wisdom S. J., Wilson R., McKillop J. H. and et. al.: Am. J. Obstet. Gynecol.; 165:1701-1704, 1991.
17. Wang Y., Walsh S. W., Guo J. and et. al.: J. Am. Obstet. Gynecol.; 165:1695-1700, 1991.
18. Davidge S. T., Hubel C. A., Brayden R. D. and et. al.: J. Am. Obstet. Gynecol.; 79: 897-901, 1992.
19. Knekt P., Aromaa A., Maatela J. and et. al.: Am. J. Clin. Nutr.; 53:283S 286S, 1991.
20. Meister A. and Anderson M. E.: Ann. Rev. Biochem.; 220:462, 1983.
21. www.http://lpi.orst.edu/infocenter/vitamins/vitaminE/e.html, p. 1-8.
22. Levi F., Pasche C., La-Vecchia C. and et. al.: Int. J. Cancer; 77:705-709, 1998.
23. Skibba J. L., Powers R. H., Stadnicka A. and et. al.: Int. J. Hyperthermia; 7(5):749-761, 1991.
24. Boxetti C., La-Vecchia C., Talamini R. and et. al.: Int. J. Cancer; 87:289-294, 2000.
25. Yu M., Garabrant D. H., Peters J. M., and et. al.: Cancer Res.; 48:3843-3848, 1988.
26. Launoy G., Milan C., Day N. E., and et. al.: Int. J. Cancer; 76.27-12, 1998.
27. www.http://lpi.orst.edu/infocenter/vitamins/vitaminc/c.html, p. 1-8.
28. Carr A. C. and Frei B.: Am. J. Clin. Nutr., 69:1086-1107, 1999.
29. Levine G., Frei B., Koulouris S. N. and et. al.: Circulation; 93: 1107-1113, 1996.
30. Hu J., Nyren O., Wolk A. and et. al.; Int. J. Cancer; 57:38-46, 1994.
31. Arivazhagan S., Balasenthil S. and Nagini S.: Med. Sci. Res., 27:527-529, 1999.
32. Stahl E. J. T., Ela S. W., Roederer M., and et. al.: Lancet; 339: 909, 1992.
33. Leedle R. A. and Aust S. D.: Lipids; 25:241-245, 1990.
34. Hall A. G.: Blood; 97:393-398, 2001.
35. Kaplan H. S.: Hodgkin's Disease. Cambridge Mass, Harvard University Press, U. K., 1983, p.p. 236-238.
36. Moison R. M., Adriaan A., Haasnoot D. and et. al.: Rad. Biol. Med.; 25:321, 1998.
37. Fisher G. L., Byers V. S. and et. al.: Oncology; 35:22-25, 1978.
38. Swain J. A., Usmar V. D. and Gutteridge J. M. C.: FEBS Lett.; 347:49-52, 1994.
39. Krachler M., Lindschinger M., Eber B. and et. al.: Biol. Trace Elem. Res.; 60:175, 1997.
40. Schicha H., Klein H., Kasperek K. and et. al.: Beitr. Pathol. Anal.; 183:245, 1969.
41. Abdullah B., Dashti H., Mathur M. and et. al.: Elements in Health and Disease. Said H. Editor, Karachi, Hamdard, 1985, p.p. 197.
42. Abdullah B., Dashti H., Hyat L. and et. al.: Metabolism of Minerals and Trace Elements in Human Disease. Abdullah M., Dashti H., Sarkar B. and et. al. Eds. Smith-Gordon Comp. Ltd., 1989, p.p. 29, 45.
43. Robberecht H. and Deelstra H.: J. Trace Elem. Electrolytes in Heal.& Disease; 8:129-143, 1994.
44. www.http://lpi.orst/infocenter/minerals/selenium/selenium.html, p. 1-15.
45. Mustacich D. and Powis G.: J. Biochem.; 346:1-8, 2000.
46. Broghamer W., Mcconnell K. J., and Blotrky A.: Cancer; 37:1384, 1976.
47. Comb G. F. and Gray W. P.: Pharmacology and Therapeutics, 79.179-192, 1998.

الخلاصة

إن نقصان الفيتامينات والمعادن في الجسم مرتبط بهبوط مناعته. وقد دلت الدراسات الميدانية عن وجود علاقة بين التغذية وحالات السرطان. وتهدف الدراسة الحالية إلى تقييم حالة التغذوية عند المصابين بسرطان الدم (بمختلف أنواعه) بدلالة مستويات البيتاكاروتين وفيتامينات (C, E, A) والألبومين والخارصين والنحاس والسلينيوم في الدم، بينما يعكس قياس الثايول الكلي (Total thiol) في الدم حالة للجهد التأكسدي.

للغرض المذكور أخذت نماذج دم من 105 مريضاً مصاباً بسرطان الدم:

27 ALL, 27 AML, 23 CLL & 28 CML

قورنت النتائج مع تلك التي تم الحصول عليها من 50 شخصاً طبيعياً بنفس العمر والجنس، وأظهرت النتائج انخفاضاً في جميع المتغيرات التي تمت دراستها عدا تركيز النحاس ونسبة النحاس إلى الخارصين (حيث كانتا أعلى بصورة ملحوظة) في دم المرضى. ولوحظ عودة كل المتغيرات المذكورة إلى مستوياتها الاعتيادية أو إلى ما يشابه ذلك بعد إكمال العلاج والوصول إلى حالة الشفاء.