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Vitamins A, E, Microelements and Membrane Lipid Peroxidation in Patients with Neoplastic Disease Treated with Calcium Antagonists and Antagonists of Receptors H_2

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Abstract. *We studied the serum levels of vitamins A, E, zinc and copper in two hundred and twenty-five subjects of both sexes. They were divided into two groups: 87 healthy subjects who served as controls and 138 patients with neoplastic disease. The patients were subdivided according to the absence (n=79) or the presence of metastatic disease (n=59). In 59 patients with cancer, who were in therapy with scavenger drugs of free radical such as calcium antagonists and the antagonists of receptors H_2, we also studied the possible effect of the same therapy on the serum levels of vitamins, on the concentrations of the microelements and on membrane lipid peroxidation. We found that membrane lipid peroxidation, evaluated from the time of in vitro formation in the blood of so-called "Heinz bodies," decreased in all patients treated with scavenger drugs. In these patients the permeability of the erythrocyte membrane was similar to the controls and the serum levels of the vitamins were equal to the levels in patients who did not receive these therapies. Zinc concentration increased while copper remained unchanged. We also studied the levels of vitamins in some organs. The results are discussed considering the role of free radicals. We underline the importance of vitamins A and E in the protection from membranous peroxidation and from free radicals and the need to consider cancer as a systemic morbid event, apart from the contingent actual location.*

Copper and zinc are involved in many biochemical processes (1) such as cellular respiration, DNA and RNA synthesis, maintenance of the integrity of the cellular membranes and sequestration of free radicals. Percivall (2) observed that the immune system requires normal levels of copper and zinc to perform several functions, however, the biochemical mechanism is unclear. Lack of copper reduces the level of interleukin-2 and the proliferation of T cells. Moreover, dietary copper is essential for cardiovascular homeostasis (3). Deficiencies of zinc and zinc-dependent immunologic dysfunctions are present in more than half of the patients with head and neck cancer (4). Further, an increase of plasma levels of copper and a decrease of zinc levels have shown up in the metastatic process (5).

Previous studies suggested that, in cancer, free radicals cause lipoperoxidation of erythrocytic membranes with increased membrane permeability, while hemoglobin is denaturalized due to the oxidative stress (5,6). Furthermore, oxidative damage was more evident in patients with neoplastic disease than in healthy subjects (7). This phenomenon can be evaluated either by calculating the time necessary for the intra-erythrocytic formation of "Heinz bodies" after induction of oxidative stress in the venous blood *in vitro* with acetylphenylhydrazine (APH), or by calculating the quantity of methemoglobin which is formed after the same treatment. In another study, we observed that therapy with calcium antagonist drugs or antagonists of receptors H_2 in cancer reduced oxidative damage (8). Calcium and receptors H_2 antagonists, which behave as antioxidant agents, have been implicated in blocking the steps of carcinogenesis.

We also observed that the presence of free radicals in the blood of cancer patients is directly influenced by antioxidant substances (9), *i.e.* vitamins A and E. These amass upon the cellular membranes, in order to protect them from free radicals peroxidation, thus their blood concentrations are greatly reduced compared with controls. The damage caused by free radicals can be blocked by scavengers of free radicals such as reduced sulfhydryl compounds, certain enzymes, selenium, microelements and vitamins A, C and E (10-12).

In non-smoking men with colon cancer, we observed that the -SH groups, the levels of glutathione in the plasma and the erythrocytes were significantly reduced compared with

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Table I. *Basic statistics of variables studied (vit. A, vit. E, Zn, Cu and age; X = mean, S.D. = Standard Deviation, E.S. = Error Standard).*

	Controls N = 87			Tumors N = 138		
	X	S.D.	E.S.	*X*	S.D.	E.S.
Vit. A	66.99	19.70	2.11	43.82	21.00	1.79
Vit. E	1375.03	393.49	42.19	1004.44	486.90	41.45
Zn	80.95	27.83	2.98	101.52	57.97	4.93
Cu	102.38	43.97	4.71	132.43	90.75	7.73
Age	51.82	17.61	1.89	67.02	11.18	0.85
	Tumors without metastasis N = 79			Tumors with metastasis N = 59		
	X	S.D.	E.S.	*X*	S.D.	E.S.
Vit. A	44.62	24.58	2.76	42.75	15.08	1.96
Vit. E	1022.00	455.98	51.30	980.44	528.52	68.81
Zn	89.20	38.19	4.30	118.00	74.11	9.65
Cu	123.61	71.02	7.99	144.23	111.46	14.51
Age	66.68	12.01	1.35	67.47	10.05	1.31

Table II. *Basic statistics of variables studied in the various tumor locations (lung, gastric, breast and colon) (X = mean, S.D. = Standard Deviation, E.S. = Error Standard).*

	Lung N = 14			Gastric N = 20		
	X	S.D.	E.S.	*X*	S.D.	E.S.
Vit. A	33.92	15.27	4.08	39.47	19.06	4.26
Vit. E	798.33	310.20	82.92	871.32	329.95	73.78
Zn	89.70	62.39	16.68	98.82	46.48	10.47
Cu	204.20	112.16	29.98	102.55	62.20	13.91
Age	64.21	9.92	2.65	68.40	9.99	2.23
	Breast N = 18			Colon N = 33		
	X	S.D.	E.S.	*X*	S.D.	E.S.
Vit. A	51.43	15.28	3.60	46.29	22.91	3.99
Vit. E	1064.81	434.76	102.47	1006.82	512.69	89.25
Zn	104.20	83.05	19.57	101.30	48.90	8.51
Cu	137.52	80.52	18.98	109.84	68.75	11.97
Age	62.00	14.22	3.35	71.64	9.23	1.61

controls (10).Vitamins A and E act synergistically at low doses. Vitamin E blocks the free radicals forming a quinone structure by contributing one electron to the lipid components of the cellular membranes (11).

Vitamin A could prevent cancer of various organs (12) by suppressing formation of hydrogen peroxide; it has been used in many trials (13-15) to reduce the oxidative stress caused by free radicals and prevent some forms of cancer or the malignant transformation of precancerous lesions.

In the present study, we evaluated the serum levels of copper, zinc and vitamins A and E in cancer patients and in healthy controls. We studied the relationship among the levels of copper, zinc and vitamins A and E, and the effects of calcium and H_2 receptors antagonists on the serum levels of microelements and liposoluble vitamins were evaluated.

Patients and Methods

Two hundred and twenty-five individuals of both sexes were prospectively studied. They were divided into two groups: 87 healthy subjects who served as controls (age range, 17-84 years; mean, 51.8 years) and 138 patients with neoplastic disease (age range, 35-89 years; mean, 67 years). The patients with neoplastic disease were subdivided according to the absence (n=79) or the presence of metastatic disease (n=59). In addition, 59 patients with neoplastic disease, who were being treated with scavenger drugs, were evaluated to determine the possible effects on the studied elements. Of these, 32 patients did not have metastatic disease and 27 did. After the study had been fully explained, the patients provided informed consent.

Vitamins A and E were simultaneously estimated in the serum by high-performance liquid chromatography (HPLC) model Chromat II System (BIO-RAD Laboratories, Segrate, Milan, Italy). The methodology has been described previously (9). HPLC displays the two peak values on the same chromatogram expressed in mg/dl.

To determine the copper and zinc levels, we used the spectrometric methodology with plasma emission reported by Roberts *et al.* (16). The values are expressed as mg/dl.

Verification of membrane lipoperoxidation. The methodology to determine peroxidation and to study the permeability of erythrocytic membranes *in vitro* has been reported previously (6,7). In all our previous studies the oxidative stress was induced by treating whole blood with APH, a substance which produces free radicals. APH increases membrane permeability of erythrocytes and "Heinz Bodies" are produced. Free radicals, in cancer, increase erythrocyte membrane permeability and predenaturate oxyhemoglobin so that erythrocyte membrane becomes more susceptible to new oxidative stress, such as that induced by APH.

We observed that the increase of permeability was significantly higher in cancer patients than in healthy donors.

Statistical analysis. The serum levels of vitamins A, E, zinc and copper were measured in the controls and patients with cancer. A possible relationship between the microelements and the vitamins was evaluated. The effects of age and tobacco abuse also were considered. The patients with tumors were studied to determine the possible effect of scavenger drugs, such as calcium antagonists and the antagonists of receptors H_2, on the serum levels of vitamins, the concentrations of the microelements and on membrane peroxidation. A p value less than 0.05 was considered statistically significant. The Mann-Whitney test, the Dunn test, the Kendall coefficient of correlation and the Kruskal Wallis test were used.

Results

Table I shows the statistical basis of the vitamin A, E, zinc, copper and age in the controls and the patients with or without metastatic tumors.

The first analysis, the Mann-Whitney test (17), permitted us to compare between the control group and tumor group

Table III. *Comparisons among the parameters in controls and cancer patients by tumor location (Dunn test, * = Significant comparisons).*

	Vit. A	Vit. E	Zn	Cu
lung → stomach	0.79	0.43	-1.87	2.88*
lung → breast	2.21	1.75	-1.10	0.98
lung → colon	1.79	1.46	-2.59	2.78*
lung → controls	5.32*	4.53*	0.93	2.82*
stomach → breast	1.57	1.46	0.81	-2.02
stomach → colon	1.04	1.21	0.02	0.41
stomach → controls	5.06*	4.82*	1.54	0.77
breast → colon	0.74	0.53	0.91	1.84
breast → controls	2.88*	2.73*	0.47	1.79
colon → controls	-4.70*	-4.10*	1.90	0.36

the levels of vitamin A, E, copper and zinc. There were significant differences in the values of vitamin A (z=-7.765 - p=0.0001) and vitamin E (z=-6 - p=0.0001). The serum values were significantly lower in cancer patients than in the controls, as previously reported (9). The level of zinc, however, was higher (z=-2.406 - p= 0.016) in the presence of cancer; the level of copper did not change significantly.

When we compared the controls with cancer patients with or without metastases using the Kruskal Wallis test (18), analogous results were obtained: vitamin A: χ^2=60.678 - p=0.0001; vitamin E: χ^2=36.258 - p=0.0001; zinc: χ^2=7.552 - p= 0.023; and copper: no significant changes (p=N.S.).

The Dunn test (19) identified significant correlations between controls and cancer patients with or without metastasis, while there were no differences within the tumors even if the tumors that did not metastasize had higher values of vitamins A and E, and the zinc values were lower compared to the group with metastases.

These findings indicate that, with the exception of copper, significant variations occur in the parameters studied in the early phase of the disease. The Kendall coefficient of correlation (18) was used to measure the eventual degree of association among the variables. The results show that in all three groups the only significant correlation was between vitamins A and E (controls: t=0.319, p=0.0001; no metastatic tumor: t=0.252 - p=0.0001 – metastatic tumor: t=0.355 - p=0.005), while there was no correlation between the vitamins and the other variables or between copper and zinc. In addition, the Kendall coefficient verified that age does not affect the parameters.

A parallel study was also performed based on the location of the tumors (lung n=14; stomach n=20; breast n=18 and colon n=33). Table II shows the mean, the Standard Deviation and the Error Standard of the parameters observed based on the location of the tumors. The correlations among the variables were also evaluated with the Kendall coefficient, which indicated that there were no significant associations in colon, stomach and breast cancers between vitamins and minerals; therefore, the variables are independent from each other. There was, however, a correlation in lung cancer between vitamins A and E (t=0.47 - p=0.01).

We further compared the parameters observed in the tumors based on the various localizations and those observed in the controls. The Kruskal Wallis test (vitamin A: χ^2=56.011- p=0.0001; vitamin E: χ^2=43.647- p=0.0001; copper: χ^2=12.971- p=0.011; zinc: p=N.S.) and the Dunn test (Table III) shows that, with regards to vitamins A and E, all comparisons between tumor location and the controls were significant; however, within tumor locations there were no significant differences. No meaningful comparison was found for zinc, which remained stable. Regarding copper, the lung value was significantly higher compared with the colon, the stomach and the control group (comparative value, Q=2.639).

In Table IV base statistics of scavenger-treated and not treated patients are shown.

The Mann-Whitney test (Table V) was used to evaluate possible differences in the parameters between treated and untreated cancer patients. The results showed that therapy did not cause important variations in the studied parameters.

The Kendall coefficient, used to analyze differences between the groups of treated and untreated patients, showed a correlation between the levels of vitamin A and vitamin E (treated patients: t=0.304 - p=0.001; untreated patients: t=0.266 - p=0.0001). The administration of scavenger therapy did not change the relationship between the two vitamins.

Table IV. *Basic statistics of variables studied in cancer patients with and without scavenger drug therapy. (X = mean, S.D. = Standard Deviation, E.S. = Error Standard).*

	Subjects under therapy (N = 59)			Subjects not under therapy (N = 79)		
	X	S.D.	E.S.	*X*	S.D.	E.S.
Vit. A	47.49	24.94	4.41	42.67	24.41	3.56
Vit. E	1035.65	443.45	78.39	1013.33	468.86	68.39
Zn	87.74	36.71	6.49	90.20	39.53	5.77
Cu	124.62	58.24	12.07	122.93	73.57	10.73

Table V. *Comparisons among the parameters studied in cancer patients with and without scavenger drug therapy (Z = Mann-Whitney test, Z_{tab}=1.96; Z_{tab}= tabular value).*

	Vit. A	Vit. E	Cu	Zn
Z	0.71	0.31	1.08	0.41
p	0.48	0.76	0.28	0.68

Table VI. *Comparisons among the parameters studied in cancer patients who did and did not receive therapy and the patients with and without metastasis (H = Kruskal Wallis test, χ^2_{tab} = 7.82; χ^2_{tab} = tabular value).*

	Vit. A	Vit. E	Cu	Zn
H	1.22	1.45	5.85	1.33
p	0.75	0.69	0.12	0.72

Table VII. *Comparisons between serum levels of copper and zinc in cancer patients and in controls by sex and tobacco abuse (Z = Mann-Whitney test, Z_{tab} = 1.96; Z_{tab} = tabular value).*

	Controls		Tumors without metastasis		Tumors with metastasis	
	Gender					
	Z	*p*	Z	*p*	Z	*p*
Zn	-0.57	0.57	-1.47	0.14	-0.62	0.53
Cu	-0.06	0.96	-1.01	0.31	-0.16	0.87
	Smoke					
	Z	*p*	Z	*p*	Z	*p*
Zn	-0.18	0.86	-1.04	0.30	-0.47	0.64
Cu	-0.17	0.87	-0.40	0.69	-0.17	0.86

The cancer patients were further subdivided based on the presence or absence of metastasis. Analysis using the Kruskal Wallis test (Table VI) showed that therapy did not result in statistical differences among the values of the vitamins, zinc and copper in the presence of metastasis.

The Mann-Whitney test was also applied to all the subjects examined, identified for gender and tobacco abuse, to determine any difference in the copper and zinc values. Table VII shows that the values of zinc and copper are not affected by gender and tobacco abuse.

Discussion

In our studies the serum level of zinc statistically increased in cancer patients compared to controls, while the copper level did not vary significantly. However, in literature the serum levels of zinc and copper are very uneven.

Magalova shows a copper increase in gastric and colorectal cancer, whereas the level remained unchanged and zinc barely decreased in breast cancer (20). According to another study, copper significantly increases in breast cancer, while zinc remains unchanged (21). In melanoma, copper is similar to that from healthy subjects, while zinc significantly increases (22). Prasad and associates observed that zinc did not decrease and copper rose in head and neck cancer (4). In oral squamous cell carcinoma, copper increases and zinc falls in male patients only (23). In cancer patients zinc decreases, while copper is usually steady (24). The zinc is quite low in gynecologic cancer, whereas copper does not differ statistically (25). In patients with breast cancer, zinc and copper significantly increase (26). Magalova *et al.* suggest that the serum levels of copper and zinc vary, according to neoplastic pathology locations (20).

In terms of the serum levels of copper and zinc in our study, we found that: i) the quantities of the two microelements are not associated with gender, age, or tobacco abuse; ii) in our patients there were no significant variations of the two elements in relation to the stage of the disease; iii) no correlation was found with the vitamins examined; iv) increased levels of zinc were found in the early stages of the disease; v) in the lung there was a significant increase in copper compared to other organs and to the general average.

It follows from our results that: i) the serum levels of vitamins A and E vastly decrease ($p<0.0001$) in the serum level of cancer patients; ii) the reduction in the serum values of the two vitamins is unrelated to gender, age and tobacco abuse; iii) in our patients there were no significant variations of the two vitamins in relation to the evolution of the disease; iv) in cancer there is a correlation between free radicals and vitamins A and E; in fact, the vitamins tend to amass upon the membrane of the cells, thus blocking the increased peroxidation due to free radicals, thereby reducing the quantity of vitamins in circulation; v) the biggest decrease of vitamins A and E in lung cancer could be due to the role played by O_2 and NO free radicals in the carcinogenesis of this organ (27, 28). In lung cancer the activity of the free radicals is clearly better related to the higher number of substances that generate free radicals: it is even more obvious if we consider the carcinogenetic substances released by the smoke of a cigarette (29).

The patients treated with the scavenger drugs did not show variations in the levels of the microelements and the liposoluble vitamins. The scavenger drugs could block radical reactions and prevent membrane lipoperoxidation, as we demonstrated (8). In fact the patients treated with scavenger drugs had a peroxidation level of the erythrocytic membrane equal to that of the controls (8).

Given that the scavenger drugs can block radical reactions and prevent membrane peroxidation, it is not understood why patients treated for many years have vitamin A and E values identical to the values of patients who were never treated with scavenger drugs.

The present results support the aspect that cancer is a systemic morbidity, involving the organism as a whole from the onset. Therefore defense mechanisms and resistance of ill organisms are very important from the preclinical stages of the disease. There are two important factors in the evolution of the illness: on one hand the damaging action of free radicals and the following peroxidative stress, on the other hand the protective activity of liposoluble vitamins.

Keeping a standard serum level of these vitamins is therefore fundamental in order to prevent and treat the illness. This is especially true in the light of the results achieved, as they draw attention to a steady impoverishment of the level of these vitamins in the various locations of the illness.

With further specific *in vivo* studies, the mechanisms of action of the scavengers on membrane permeability, levels of liposoluble vitamins and free radicals in cancer may be clarified.

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