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## Erythrocyte Membrane Fatty Acid Composition in Cancer Patients

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Essential fatty acids (EFA) have an important role in complex metabolic reactions. The metabolism of essential polyunsaturated fatty acids (PUFA) appears to be one of the critical targets in the complex metabolic stages that lead to, or are associated with cancer. The goal of our research was to analyze the erythrocyte specific types of membrane fatty acid content, level and distribution in cancer patients as compared to non-cancer patients. Changes in fatty acid composition may affect different aspects of cell structure and function, including proliferation. Analyses of RBCs membrane fatty acids were performed for 255 patients with different types of cancer (breast, prostate, liver,

pancreas, colon, and lung), 2,800 non-cancer patients and 34 healthy volunteers. Our research study demonstrated a lower level of stearic acid and an increased content of oleic acid in RBC of cancer patients in comparison with control and non-cancer patients. According to the results of this investigation, the ratio of Eicosa pentaenoic acid (EPA) and Decosa hexaenoic acid (DHA) to Alpha-linolenic acid (ALA) may be useful to estimate PUFA imbalances in cancer patients. EPA and DHA acid may be recommended as supplementation and in addition to current therapy during cancer treatment.

*Key words: Erythrocytes, Fatty acids, Cancer*

**M**any studies have demonstrated that dietary fats may affect the incidence of cancer and markedly influence tumor development. Dietary fatty acids (FA) may modulate various important membrane parameters such as: membrane associated receptors, tumor antigens, prostaglandin synthesis membrane potential and membrane fluidity. Essential fatty acids (EFA) have an important role in complex metabolic reactions. The metabolism of essential polyunsaturated fatty acids (PUFA) appears to be one of the critical targets in the complex metabolic stages that lead to, or are associated with cancer. A decrease in PUFA level is one of the biochemical abnormalities that strongly correlate with cell transformation (1, 2). The loss of specific PUFA is either due to changes in enzyme activity or decreased concentration of substrates that may be accompanied by an array of changes that may promote the development of malignancies.

Neoplastic tissues exhibit differences in the lipid metabolism as compared to normal cells. An elevation in

distinct lipids (3) and a decrease in PUFA levels (4) have been strongly correlated with cell transformation.

Tumor cells also tend to have a decreased activity of EFA desaturase enzymes (5). The activities of the desaturase enzymes are under hormonal and nutritional control, but the molecular mechanisms of this control have not been totally elucidated (6). Especially, the delta -6-desaturase enzyme appears important, since its activity determines the tissue level of polyunsaturated acids, such as gamma-linolenic acid (GLA), dihomogamma-linolenic acid (DGLA) and the series 1 eicosanoids.

Many types of tumor cells exhibit a decreased rate of lipid peroxidation compared to that seen in normal cells, as a result of lower content of PUFA. A high rate of cell division correlates inversely with the rate of lipid peroxidation in both normal and tumor cells (7). The extreme importance of lipid peroxidation products (LPP) in cell proliferation is now acknowledged (8, 9). LPP were previously considered as components that should be prevented by antioxidants, LPP are now recognized as metabolites involved in the control of tumor growth and determinants of tumor sensitivity to anticancer agents. Incorporation of omega-3 PUFA in tissues provides excellent substrates for lipid peroxidation.

Animal studies indicate that omega-6 fatty acids promote tumor growth while omega-3 fatty acids inhibit tumor development. For example, a fish oil-derived concentrate enriched in eicosapentaenoic (EPA) and

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docosahexaenoic acid (DHA) as ethyl esters inhibits the formation and growth of aberrant crypt foci in rat colon (10). Omega-3 fatty acids decrease tumor incidence and/or number and slow the development of transplanted tumors (11). Furthermore, the mixture of linoleic acid (LA) and GLA (12-14) or of EPA and DHA (15) has been administered to rats with transplanted mammary tumors with favorable results.

The evidence of the relationship between cancer and FA with special reference to the omega-3 FA and the different mechanisms for the effect of dietary FA and alterations of lipid and fatty acid metabolism in cancer patients have been analyzed in many studies (16-21). Epidemiological evidence indicates that dietary patterns are important and fat content is one of components correlated to increased cancer incidence. The evidence for and against each of the suggested mechanisms, through which omega-3 fatty acids may influence cancer has been analyzed previously (22). The evidence appears to be strongest for three mechanisms, lipid peroxidation, modification of the eicosanoid synthesis and enhanced immunity. Other potential mechanisms reported (22) include, membrane fluidity, intercellular communication, hormone secretion and serum lipid levels.

The connection between dietary fat and tumor promotion may be the result of the influence of specific fatty acids on lipid peroxidation and prostaglandin E2 production. In addition omega-6 fatty acids may increase the production of the immunosuppressive prostaglandin E2 through the arachidonic acid pathway (23, 24). Because of the ability of omega-3 PUFA to decrease prostaglandin E2 synthesis, several reports described the beneficial effects of dietary omega-3 fatty acid in reducing of carcinogenesis (25, 26).

In a pilot study (27), which was designed to evaluate whether PUFA's are associated with human prostate cancer, the level of erythrocyte membrane omega-3 and omega-6 fatty acids were determined for prostate cancer patients. It was demonstrated that linoleic acid and total omega-6 fatty acids were associated with an increased risk of prostate cancer.

Several studies (28, 29, 37-39) have demonstrated changes in ratio of stearic to oleic acid, desaturation of stearic acid and changes in the proportion of stearic to oleic acid in lipid layer of erythrocytes of cancer patients. The degree of saturation in the red cells was higher in patients with benign breast tumor than with malignant tumor (28, 29). It was also established that a relationship exists between the degree of lipid unsaturation, the tumor spread (stage of disease, metastases into the regional lymph nodes), and patient survival. Decrease in the level of PUFA's was observed in lung cancer patients (30).

In the report of the Multiple Risk Factor Intervention

Trial (MRFIT) study (31) and review (32) it was shown that oils rich in omega-3 FA reduce tumor incidence, growth and metastatic spread. This action may occur by various means: As for example, omega-3 FA may reduce tumor cell growth or stimulate tumor cell loss and moreover, do not show any tumor-promoting activity in comparison to omega-6 FA.

A large prospective study reported that PUFA's inhibited some steps in colon carcinogenesis (33). According to this research, omega-3 PUFA are promising molecules in cancer prevention and may potentiate any cancer treatment. The effect of omega 3 PUFA on tumor growth might as well depend upon background levels of omega-6 PUFA and antioxidants (34). Many factors can change enzymatic and nonenzymatic activities involved in the metabolism of EFA. For example, metals (iron, zinc, copper), vitamins (A, B6, C, E) and EFA themselves are all nutrients that can activate or interfere with known biochemical pathways.

The question of whether the fatty acid composition of erythrocyte membranes reflects the fatty acid composition of other tissues remains unexplored. Some earlier studies supported hypothesis that the fatty acid composition of membrane differed from tissue to tissue (35), while other studies have found a positive correlation between fatty acid composition in erythrocytes and muscle cell membranes FA. The fatty acid composition in RBCs has been analyzed previously. Our study allowed a more comprehensive analysis of the RBC FA based on the larger statistical data we had available.

The results of our research may be useful to identify the dietary fatty acids which can be measured in clinical studies to improve the nutritional and disease status of cancer patients.

## Methods

Membrane fatty acid composition was analyzed by the Folch technique (36), with slight modifications. The erythrocyte fraction, which was obtained from blood by centrifugation, was lysed by addition of a low-molarity solution. Membrane suspension was separated from cytoplasm, protease, and other cell components by centrifugation. Analysis of fatty acids and fatty acid composition of RBCs was performed by lipid extraction and analysis by gas chromatography.

We analyzed the specific types of fatty acids in the membranes of RBCs in cancer patients, non-cancer patients and healthy volunteers. Analyses of RBCs membrane fatty acids were performed for 255 patients with different types of cancer (breast, prostate, liver, pancreas, colon, and lung), 2,800 non-cancer patients and 34 healthy volunteers.

Table 1. Composition of RBCs fatty acids in patients with breast cancer

Age, sex	CEA	w6/w3	LA	DGLA	AA	GLA	ALA	EPA	DH A	w3 total	Nervonic	Oleic	Palmitic	Stearic	s/o ratio
73.f	3.9	5.33	11.1	1.5	13.9	0.03	0.28	1.2	3.5	5	1.2	10.1	23.8	11.4	1.13
61.f	0.7	2.59	8.2	2.3	11.4	0.03	0.26	1.1	7.1	8.5	0.92	12.9	17.4	12.3	0.95
45.f	9.9	5.57	12.3	1.1	13.5	0.04	0.2	2.24	2.4	4.8	0.46	16.7	23.6	13.7	0.82
42.f	134	7.70	13.8	1.4	13.1	0.1	0.22	0.67	2.8	3.7	0.5	13.8	23.6	13.2	0.96
45.f	18.2	5.34	8.4	1.2	11.5	0.06	0.54	0.62	2.8	4	0.09	12.2	20.9	11.8	0.97
47.f	2.5	7.89	11	1.1	15.4	0.024	0.13	0.36	3	3.5	0.7	16	18.2	14.7	0.92
45.f	8.3	9.50	11.6	1.9	12.1	0.05	0.1	0.2	2.4	2.7	0.4	15.3	20.9	14.5	0.95
49.f	3.8	11.07	11.3	1.3	14	0.13	0.17	0.34	1.9	2.4	0.72	12.9	25.3	13.1	1.02
57.f	9.1	2.72	10	1.3	8.5	0.09	0.42	1.6	5.3	7.3	1	13.2	22	14	1.06
51.f	0.8	4.10	10.82	1.4	14.4	0.06	0.16	1.13	5.2	6.5	0.1	13.6	21.4	15.1	1.11
78.f	0.7	7.49	11.1	1.6	14.1	0.08	0.18	0.41	3	3.6	0.39	14.6	21.8	16.3	1.12
82.f	0.7	5.87	10.6	1.7	13.8	0.02	0.22	0.36	3.9	4.5	0.6	13.1	20.9	14.3	1.09
43.f	24.3	2.74	13.51	2	8.73	0.04	0.4	1.85	6.6	8.9	1.27	12.4	21.9	14.4	1.16
53.f	0.8	3.21	9.9	1.4	14.5	0.02	0.1	1.1	6.9	8.1	1.19	15.6	17	14.6	0.93
42.f		5.48	11.3	1.2	15	0.03	0.12	0.3	4.6	5	1.9	11.6	20.4	16.1	1.39
39.f		9.25	12.1	2.9	15	0.05	0.22	0.33	2.7	3.3	1.3	15.4	17.3	16	1.04
55.f		5.04	12.3	0	12.8	0.07	0.2	0.6	4.2	5	0.9	13.5	23.5	13.7	1.01
45.f		4.86	11.8	2.1	15.2	0.06	0.4	0.6	5	6	1.3	11.4	17.8	14.5	1.27
49.f		8.18	10.6	1.7	15.5	0.02	0.22	0.38	2.8	3.4	0.4	14.5	22.6	14.6	1.01
58.f		5.98	10.4	1.5	16.8	0.04	0.28	0.63	3.9	4.8	0.7	14.8	20	15.4	1.04
44.f		8.09	12.6	1.2	13.1	0.04	0.21	0.32	2.8	3.3	0.4	13.5	24.6	15.1	1.12
46.f		8.55	13.1	1.8	16.7	0.05	0.18	0.42	3.1	3.7	0.78	15.8	19.3	13.8	0.87
66.f		9.60	13.3	1.7	16.9	0.06	0.23	0.7	2.4	3.3	0.6	12.6	21.4	12.9	1.02
67.f	1.3	5.03	9.56	2.1	15.1	0.03	0.17	0.65	4.5	5.3	0.07	13.9	20.5	14.9	1.07
55.f		7.45	9.8	1.3	14.6	0.09	0.13	0.43	2.9	3.5	0.7	10.5	20.9	12.3	1.17
55.f		3.81	8.54	1.8	15.5	0.08	0.16	0.93	5.7	6.8	0.49	13	20.4	14.5	1.12
70.f		4.66	9.3	1.5	12.1	0.06	0.2	0.33	4.4	4.9	2.4	12	21.2	11.3	0.94
54.f	1	5.69	12.2	1.8	14.7	0.04	0.13	0.62	4.3	5.1	0.8	14.1	20.7	14.8	1.05
58.f	18	5.64	10.4	1.6	15.3	0.06	0.21	0.64	4	4.9	0.8	15.1	21.6	15.2	1.01
53.f	1.4	5.71	11.2	2.1	17.4	0.09	0.09	0.8	4.5	5.4	1.1	12.4	18.2	14.9	1.20
52.f	2.5	8.06	12.3	1.5	14.7	0.02	0.18	0.36	3	3.5	0.6	14.8	18.1	14.8	1.00
77.f	1.9	4.42	9.5	1.3	12.9	0.1	0.2	0.59	4.6	5.4	1.2	13.7	23.2	12	0.88
44.f	4.9	4.74	10.4	1.6	12	0.17	0.2	0.7	4.2	5.1	0.6	12.7	26.4	13	1.02
73.f	1.4	4.79	10.2	1.5	11.7	0.13	0.27	0.84	3.8	4.9	0.5	16.6	24.8	13.1	0.79
61.f	1.1	4.71	11.2	1.5	14.3	0.08	0.17	0.48	5.1	5.8	0.3	14.3	20.1	14	0.98
48.f	6	3.90	11.7	2	14.7	0.06	0.19	1.6	5.5	7.3	0.3	13	17.6	15.3	1.18
57.f	2.4	7.38	12.1	1.4	14.5	0.04	0.2	0.2	3.4	3.8	1	14.4	18.6	16.1	1.12
76.f	2.5	3.67	8.1	1.2	13.6	0.12	0.17	1.3	4.8	6.3	0.24	15.8	23.9	14.9	0.94

Data presented in tables show the clinical data, which were analyzed for each patient and healthy control: level of PUFA omega-6 and omega-3, level of monounsaturated and saturated fatty acids in RBCs membrane of cancer patients and level of tumor markers CEA or PSA.

### Results

We analyzed the level of different FA in RBCs, which were normalized on the total amount of fatty acids in membrane, and the concentration of the different FA in RBCs in units of the percents of standard.

For statistical analysis we used multiple regression

analysis with correlation matrixes, non-parametric Mann-Whitney test and t-test.

For estimation of the status of the monounsaturated, polyunsaturated and saturated FA in RBCs of cancer patient, non-cancer patients and healthy volunteers, the composition of oleic FA, stearic FA, PUFA, level of omega-3 and ratio omega6/omega3 in RBCs membrane was analyzed.

The most interesting findings from the analysis of the distributions of different FA in RBC membrane of cancer, non-cancer patients and healthy volunteers were that the level of oleic acid was higher in cancer patients in comparison with non-cancer patients and healthy volunteers. The level of stearic acid was lower for cancer patients, while the level of saturation index was lower in cancer and non-cancer patients and composition of the polyunsaturated FA DHA and EPA was changed for cancer patients.

Figure 1 shows distribution of the oleic fatty acid (the most common monounsaturated FA) in RBCs of cancer and non-cancer patients. Oleic acid constitutes about 15% of the fatty acids in erythrocyte membranes and the presence of the double bond in the center of the molecules helps to govern the fluidity of the membrane matrix. Distributions presented in Figure 1 show higher level of oleic acid in RBCs

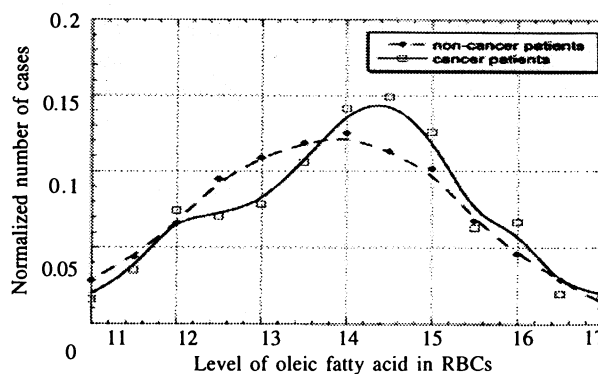
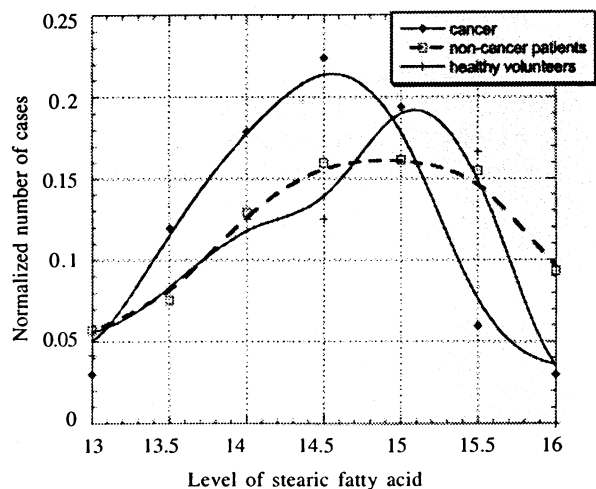


Figure 1. Distribution of the oleic fatty acid in RBCs of cancer and non-cancer patients.

of cancer patients in comparison with non-cancer patients (Mann-Whitney test,  $p < 0.05$ , medians are 13.9 for cancer patients and 13.5 for non-cancer patients).

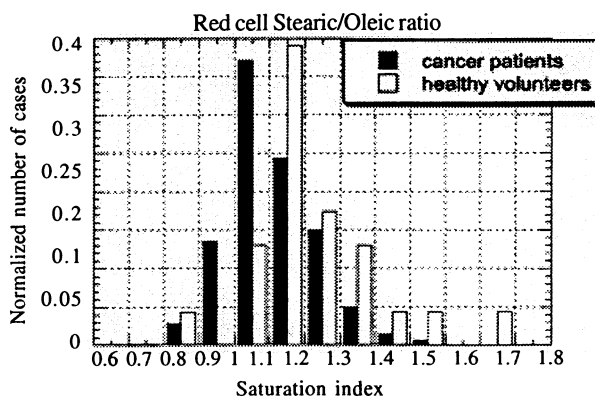
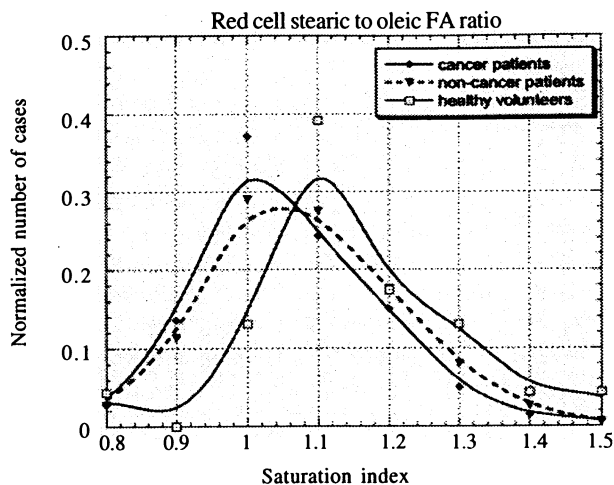
Distributions of the stearic fatty acid (the most common saturated FA SFA in of cancer patients, non-cancer patients and healthy volunteers are presented in Figure 2. Levels of stearic FA were lower for cancer patients in comparison with non-cancer patients and healthy volunteers and statistical analysis proved significant difference between medians at the level  $p < 0.05$ . The results of analysis demonstrated the increased level of oleic acid



**Figure 2.** Distribution of the level of stearic FA in RBCs of cancer patients, non-cancer patients and healthy volunteers.

and decreased level of stearic fatty acid in the lipid layer of erythrocytes in cancer patients.

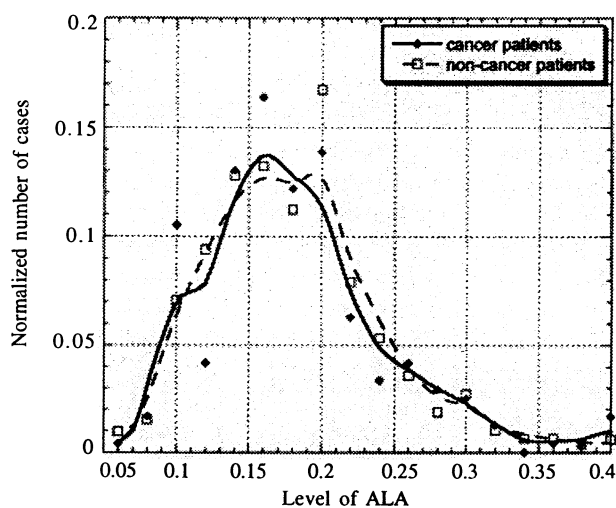
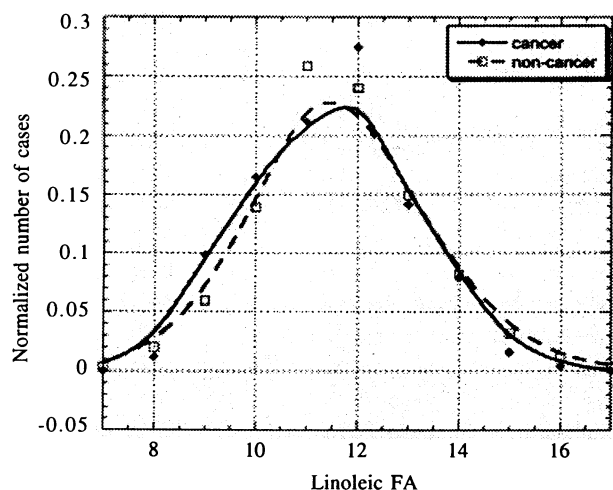
We also analyzed level of RBC saturation index for cancer patients, non-cancer patients and healthy controls. The saturation index (SI) in red blood cell membrane is the ratio of stearic acid to oleic acid. A reduction of the SI was observed previously in patients with breast cancer and other cancers in several studies (37-39), but not in all case-control studies. The role of erythrocyte membrane steric to oleic acid ratio as a marker of malignancy is still unclear. Unsaturated FA's may enhance membrane fluidity in contrast to saturated ones. In several studies, a high SI in plasma phospholipids was observed to be protective against cancer (40-42). In study with animals the stearic acid desaturation in red blood cell membranes of rats during the induction of colorectal tumors was suggested as an adaptive mechanism by changing the membrane fluidity (43). The change in membrane fluidity is an important parameter and is associated with increased metabolic rate of the lipid-dependent enzymes (44). The saturation index in three different populations is presented at Figure 3.



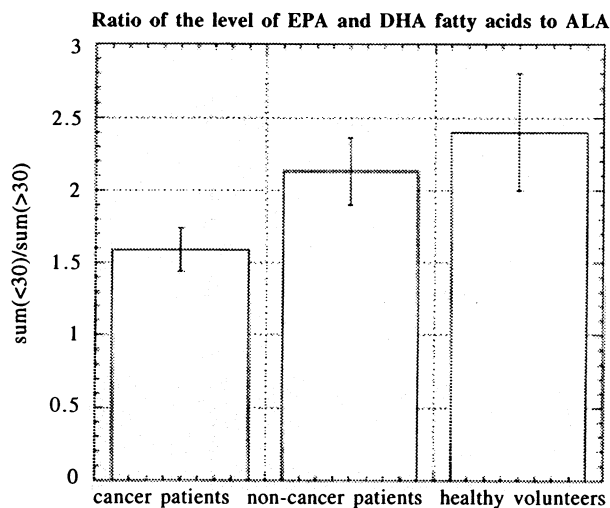
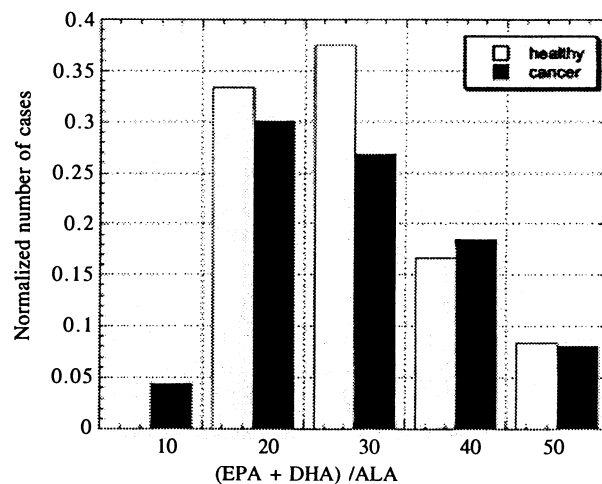
**Figure 3.** Distribution of the ratio stearic to oleic FA in RBCs of cancer patients, non-cancer patients and healthy volunteers.

We found that for cancer patients the mean and SD of the stearic to oleic acid ratio in erythrocyte membranes were  $1.05 \pm 0.12$  compared to mean and SD  $1.16 \pm 0.14$  for healthy patients and  $1.08 \pm 0.13$  for non-cancer patients. The percentage of cancer patients with levels of SI lower than 1.0 consisted of 53% in comparison with 18% for healthy volunteers.

The differences between group means for these three distributions were significant ( $p < 0.05$ ), but SI distributions for cancer patients and non-cancer patients had high levels of variability and overlapping. The saturation index demonstrated difference between healthy volunteers and patients, but is not useful as a marker of malignancy. The level of polyunsaturated fatty acid was compared in these three groups. We found no difference between level and ratio of erythrocyte PUFA's for patients with cancer and patients with other types of diseases. Levels of essential fatty acids LA (linolenic acid) and alpha-linolenic acid (ALA) did not demonstrate any difference in distributions (Figure 4).



**Figure 4.** Distribution of alpha-linolenic and linoleic fatty acids in RBC membrane of cancer and non-cancer patients.



**Figure 5.** Distribution of the ratio of EPA and DHA to ALA for cancer patients and healthy volunteers.

Since after absorption, PUFA's undergo desaturation/saturation and chain elongation/shortening reactions, we analyzed if there were changes in the levels of PUFA's due to changes in enzyme activity. The distribution of the ratio of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) to ALA and ratio of the integral of these distributions for values greater than 30 and less than 30 are presented at Figure 5.

The distributions and ratios demonstrated difference between levels of derivatives of ALA for cancer patients. Ratios in Figure 5 (bottom), calculated as the ratio of area under the curve for values greater and lower than 30, were  $1.59 \pm 0.15$  for cancer patients,  $2.13 \pm 0.23$  for non-cancer patients and  $2.4 \pm 0.4$  for healthy volunteers. Composition of RBCs showed decreased level of polyunsaturated omega-3 fatty acids with high level of unsaturation DHA

and EPA (fatty acids containing 5 and 6 double bonds) in RBCs of cancer patients. The level of parent essential fatty acid ALA and the distribution of ALA in RBCs of cancer patients were similar to distribution in ALA in RBCs of healthy volunteers and non-cancer patients (Figure 4). These results may indicate that the possible reason for differences in level of proportion between ALA, EPA and DHA fatty acids in RBCs of cancer patients is due to the decreased level of desaturase enzyme activity.

Our data shows that membranes of red cells of cancer patients have lower level of PUFA with 5 and 6 double bonds are consistent with cells experiments and epidemiological studies. Decreased level of PUFA in erythrocytes of cancer patients was found in many epidemiological studies. According to in vitro experiments the polyunsaturated FA DHA and EPA containing 5 and 6

double bonds demonstrated the possibility of selectively killing tumor cells. Our results also suggest that the membrane of red blood cells of cancer patients have the decreased levels of highly polyunsaturated FA DHA, EPA and the treatment of cancer patients with polyunsaturated omega-3 fatty containing 5 and 6 double bonds may have potential clinical usefulness.

We also tried to determine if the composition of RBCs fatty acids change with the progression of the disease; in order to characterize the state of the disease we used two tumor markers prostate specific antigen (PSA) and carcinoembryonic antigen (CEA). Comparison of the level of RBC membrane fatty acids for cancer patients with values of CEA and PSA in serum did not demonstrate a significant level of correlation. Neither age of patient or state of disease defined by the level of tumor markers; had a significant correlation with the erythrocyte content or level of fatty acid saturation.

## Discussion

The goal of our research was to analyze the erythrocyte membrane fatty acid content, level and distribution in cancer patients as compared to non-cancer patients and healthy subjects. Changes in fatty acid composition may affect different aspects of cell structure and function, including proliferation.

Our research study demonstrated a lower level of stearic acid and an increased content of oleic acid in RBC of cancer patients in comparison with control and non-cancer patients. These changes may be due to the decrease activity of the delta 9 desaturase (Delta9-d) enzyme.

This study also showed a decreased proportion of PUFA such as DHA and EPA in relation to ALA in the membranes of RBC of cancer patients. The decreased level of polyunsaturated fatty acids with 5 and 6 double bonds may be due to a decreased enzyme delta-6 desaturase activity during development of pathological condition, as there was no change in contribution of essential fatty acid ALA.

According to the results of this investigation, the ratio of EPA and DHA to ALA may be useful to estimate PUFA imbalances in cancer patients. EPA and DHA acid may be recommended as supplementation in addition to current therapy during cancer treatment. As was shown in previous research (45), low doses of these FA do not induce harmful modifications of oxidative cell metabolism. EPA treatment increased EPA and DHA content in cell membranes. DHA treatment mainly increased DHA cell membrane content. Both treatments decrease omega-6/omega-3 ratio without modifying the unsaturation index.

## Resumen

Los ácidos grasos esenciales tienen un rol importante en las reacciones metabólicas complejas. El metabolismo de los ácidos grasos esenciales poli-insaturados parece ser uno de los blancos críticos en las etapas metabólicas complejas que producen o se asocian con cáncer. La meta de este estudio fue analizar el contenido específico de ácidos grasos en la membrana de los eritrocitos, la proporción y distribución en pacientes de cáncer y en pacientes sin cáncer. Los cambios en la composición de ácidos grasos pueden afectar diversos aspectos de la estructura y función de celular, incluyendo proliferación. Se hizo un análisis en 255 pacientes con diferentes tipos de cáncer (mama, próstata, hígado, páncreas, colon y pulmón), 2,800 pacientes sin cáncer y 34 voluntarios saludables. Este estudio demostró un nivel mas bajo de ácido esteárico y un contenido de ácido oleico aumentado en las membranas de los eritrocitos de pacientes de cáncer al compararlos con los controles y pacientes sin cáncer. De acuerdo con estos resultados, la proporción de ácido eicosapentaenoico (EPA) y ácido docosahexaenoico (DHA) a ácido alfa-linolénico (ALA) puede ser útil para estimar los desbalances en ácidos grasos poliinsaturados (PUFA) en pacientes de cáncer. La suplementación con EPA y DHA podría ser recomendada para corregir los desbalances en ácidos grasos como una medida adicional en el tratamiento actual de cáncer.

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