Detection of Energy Metabolism Level in Cancer Patients by Fluorescence Emission from Serum

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Abstract

Changes in fluorescence emission of the serum from patients with various cancers are characterized. The measurement of fluorescence emission of serum in the UV-visible range allows by estimation of NAD(P)H levels to monitor the alterations in energy metabolism, resulting from changes in the level of coenzymes in serum associated with neoplastic diseases.

Introduction

Over the past years, fluorescence spectroscopy, a method that is several orders of magnitude more sensitive and more selective than absorption-based techniques, has been used to characterize physicochemical properties of biomolecules that exhibit fluorescence in cells, tissues and in serum. Consequently, fluorescence spectroscopy of biomolecules has been used to characterize cell metabolic pathways and to discriminate pathological conditions of cells, tissues and organs from their normal state.[1-6] Native fluorescence emission and excitation spectra of virus-infected human keratinocytes, carcinoma cells, and normal human keratinocytes were shown to differ in the intracellular metabolic state of reduced nicotinamide adenine dinucleotide (NADH).[7] It was suggested that the observed differences were due to an increased proportion of bound mitochondrial NADH in the cancer and virus-infected cells.

In the field of diagnostic oncology, studies indicate that native fluorescence properties of tissue can be used to distinguish normal from malignant conditions in breast, cervix, colon, and bronchus samples.[6,8-10] Measurements of emission intensity or spectral ratios of emission intensity (for example at 340 and 440nm) under UV light excitation were shown to statistically differentiate normal from malignant tissues.

In a series of studies, a fluorometric screening method was established for the analysis of the emission of serum to detect patients with tumors and chronic diseases.[11] The ultraviolet fluorescence emission spectra of serum (mostly protein content) from healthy persons and of serum from cancer patients, frequently exhibited different curve shapes.

In addition to the complexity of observing differences in emission from different fractions in serum, fluorescence of native serum can be attributed to a variety of molecules including tryptophan, tyrosine, phenylalanine, NADH, pyridoxal phosphate, bilirubin, flavin-adenine dinucleotide (FAD) and others.[12,13] The fluorescence associated with these molecules is defined by their concentration and distribution as well as the photo-physicochemical properties of their environment.

We have begun a study of detecting metabolic dysfunctions by fluorescence in human serum. Our results reported here for cancer allow us to differentiate normal from abnormal metabolic behavior in human functioning and may lead to improvement of human functioning through detection of such disorders.

Experimental Procedures
Preparation of Samples

Baseline, normal blood samples were obtained from healthy staff volunteers. The experimental samples of serum were obtained from patients, which were clinically

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diagnosed with cancer. Serum was separated from blood by centrifugation at 3500 rpm for 15 minutes. Most serum samples were obtained from individuals who had fasted overnight to exclude the effect of fluorescence emission of drugs and vitamins. Some samples were stored frozen at -20˚C for up to one month and measured after this period. To analyze the effect of freezing serum on fluorescence emission, several samples from healthy volunteers were measured before and after freezing for one month, and it was shown that the emission of serum did not change.

All specimens were diluted with PBS (phosphate buffered saline) using a ratio of 1:20 to measure the emission in the range of absorption less than 0.1 at the excitation wavelength, which allowed measuring the fluorescence in the region where there is the linear relationship between fluorescence and concentration. For calibrating the instruments we used several standards: rhodamine B, [Ru(bpy)3]2+ in EtOH : MeOH (4:1) solution, and a composite of normal serum solutions from several staff volunteers.

Procedure for Measurements

For each specimen and solvent, the fluorescence spectra were run using a SPEX spectrofluorometer (sensitivity 4000:1, double-grating spectrophotometers). The light source was a 125 W xenon lamp. The devise had two double-grating monochromators, one for excitation and another for emission. The emitted photons were measured with a Hamamatsu R928 photomultiplier tube in units counts per seconds, cps. Test solutions were placed in quartz cells, and the measurements were obtained with a 90-degree angle between the beam of excitation and the emission light path. Excitation wavelengths ranged from 315 nm to 340 nm and emissions were scanned from 330 nm to 600nm. The background curves for the solvent (PBS) were measured each time before measurements of the diluted serum. The solvent fluorescence spectra were subtracted from the serum fluorescence spectra to remove background effects.

Spectroscopy

Irradiation of the samples at wavelengths from 300 nm to 340 nm gave rise to emission in the 350 nm to 600 nm region coinciding with the respective absorption and emission of NAD(P)H, along with other contributing molecules. The excitation wavelength was chosen to exclude the effect of protein emission, which has a very intense peak in the range of 280 nm to 320 nm. Excitation of samples at wavelengths of 325 nm and 340 nm gave better resolution for NAD(P)H, the principle emitting contributor as will be shown below, and other fluorophore emissions in the visible range, since tryptophan, which absorbs at 315 nm, does not have any significant absorption in this range.

The Method for Estimating the Level of Serum Emission for Patients with Metabolic Disorders

Fluorescence emission curves for 40 healthy volunteers were measured to establish a normal range. Only serum from fasting volunteers was used for the analysis of emission range variation, as serum of non-fasting volunteers showed significant emission from vitamins (especially vitamin B_6) and drugs on the level of serum emission in UV-visible range. Average curves for healthy volunteers with standard deviations (SD) were calculated for excitation wavelengths of 315 nm, 325 nm and 340 nm.

To find the differences between serum emission for patients with different metabolic disorders and healthy volunteers, the average normal range (mean ±1 SD) was compared with measurements of serum emission from patients. The following parameters were used for the comparison: the maximum intensity of emission at 450 nm and the ratio of intensities at 450 nm and 400 nm.

Results
Metabolic Disorders

The measurements of the serum emission from patients were grouped in several different sets according to the clinical diagnosis and symptoms: cancer, thyroid disorder, diabetes and chronic fatigue syndrome (CFS). All of these diseases are associated with different types of metabolic abnormalities. Part of the patients' data representing the common diseases seen at the Center is presented in **Table 1** (p.12) (column 3 represents data of the decreased level of serum emission at 450 nm in comparison with average normal value).

A description of the analyses of the serum emission for first of these groups (cancer patients) compared to the normal group is presented below.

Comparison of Normal Subjects and Cancer Patients

Data from cancer patients were grouped by types of cancer. For many cancer patients the fluorescence emission curves were different from the average normal and were characterized by a decreased level of serum emission in the 430 nm to 500 nm range. **Figure 1**, (p.13) shows examples of serum emission for three cancer patients (lung cancer, pancreatic cancer and sarcoma) in comparison with the average normal curve for excitation 315 nm. The summary of the characteristic parameters of serum emission for patients with different types of cancer (breast cancer, lung cancer, prostate cancer, chronic lymphocytic leukemia and others) is presented in **Table 2**, (pp 14-15).

The values in the columns describe the following parameters for each patient:

* the intensity in counts per second (cps) of serum emission at 450 nm at excitation 315 nm (emission of NAD(P)H);

* percentage difference in the emission intensity of NAD(P)H (maximum intensity at 450 nm) for cancer patients in comparison with the average emission intensity at 450 nm for healthy volunteers;

* characteristics of patients and diagnosis.

Figure 2, (p.13) shows the frequency distribution of the measured intensities at 450 nm (fluorescence of NAD(P)H) for 40 healthy volunteers and for 150 patients with cancer normalized on the total number of cases.

The fit of data for these curves was estimated using the Least Square's method for 4th order polynomial function for data analysis and graphed with Kaleidagraph software. The frequency curves were fitted with correlation coefficient 0.98 for serum emission from healthy volunteers and 0.94 for serum emission from cancer patients.

The distribution in Figure 2 is bimodal for cancer patients. Two peaks are found, one at 1.1×10^6 and the other at 1.7×10^6. For the cases evaluated, breast cancer, lung cancer and leukemia tend to fall into the lower intensity domain whereas those with prostate cancer give higher intensity values.

The sensitivity of the method for cancer was defined as the percentage of patients with disease, whose level of serum emission fell below the reference value (RV), which was chosen as intersection of the two frequency distributions. The percentage of patients with their level of emission less than the reference value was 48% of all patients with cancer. We also calculated the percentage of patients with different types of cancer, whose level of fluorescence serum emission was below the average normal emission. The data indicated that 70% of patients with lung cancer, 65% of patients with chronic lymphatic leukemia, 55% of patients with bladder, ovarian, colon and pancreatic cancer, 50% of patients with breast cancer and 34% of the cases with prostate cancer had more than a 10% decrease in the level of serum emission intensity at 450 nm.

Figure 3, p.16 shows ratio of the emission intensity at 400 nm compared to emission intensity at 450 nm. For healthy volunteers, ratio of I450/I400 was in range 1.3-1.6 for 86% of cases; for cancer patients I450/I400 was less than 1.3 for 63% of cases.

Table 1. Serum emission at 450nm for patients with different types of metabolic dysfunction.

Age, sex	Level of emission at 450nm/10^{-6}	Decrease from average normal (%)	Diagnosis
32,f	1.19	35	cancer ovarian, uterus
62,m	1.34	27	cancer neoplasm
55,f	1.24	32	breast cancer
48,m	1.31	28	thyroid disorder
63,f	1.10	40	thyroid disorder, hormone imbalance, hypertension
71,m	1.46	20	hypertension, diabetes-mellitus, prostate chronic, upper respiratory infection
50,f	1.42	23	thyroid disorder, depression, pain, urticaria, yeast, systematic candida
71,m	1.01	45	chronic fatigue syndrome (CFS), yeast, candida
13,f	1.20	34	gastritis, headache, allergic Rhinitis/Hay fever
24,m	1.15	37	thyroid disorder, myositis
37,f	1.39	24	pain, arthritis, allergy
3,m	1.03	44	autism, fatigue
47,m	0.99	46	cancer pancreas
82,m	1.48	19	chronic fatigue syndrome, food allergy
13,m	1.11	39	pain extremity, fatigue
71,f	1.44	21	Coronary-artery disease (CAD), fatigue
59,f	1.43	22	asthma, depression, hypothyroidism
82,f	1.31	28	systematic candida, thyroid disorder
47,f	1.19	35	cancer ovarian, uterus, cervix
42,m	1.40	23	chronic fatigue syndrome, food allergy
38,m	1.30	29	pain, gastritis, capral tunnel syndrome
72,m	1.49	19	cancer prostate, coronary-artery disease, fatigue, arthritis
36,f	1.12	39	thyroid disorder, upper respiratory infection
46,m	1.21	34	hepatitis C unspecified
46,m	1.33	27	hepatitis C unspecified
42,f	1.33	27	allergy food, systematic candida
31,f	1.24	32	hypothyroidism, food allergy,
25,m	1.25	32	cancer lung, cancer neoplasm
11,m	1.36	26	hypertension, lipoma
19,m	1.27	31	vasculitis
18,f	1.40	24	fatigue, yeast, systematic candida
59,f	1.27	31	tinnitus, fatigue, food allergy
37,m	1.19	35	fatigue, food allergy
22,f	1.20	34	lupus, tendonitis, arthritis,
31,f	1.35	26	headache, tension, gastritis, fatigue
40,m	1.26	31	cancer colon, cancer liver
70,m	1.19	35	chronic lymphocytic leukemia
52,f	1.44	21	tendonitis, fatigue, arthritis
40,f	1.11	39	myositis, yeast, systematic candida, food allergy
29,m	1.18	35	fatigue, yeast, systematic candida, food allergy
35,f	1.37	25	Chronic fatigue syndrome, tendonitis
45,f	1.44	21	fatigue, CFS, allergy food
16,m	1.32	28	fatigue, pain, gastritis, dermatitis
25,m	1.08	41	somatic dysfunction,
53,f	0.98	47	dermatitis, fatigue, food allergy, yeast, systematic candida
2,m	0.96	47	obesity-overweight, anxiety, fatigue
23,f	1.29	29	Epstein-Barr infection, cytomegalic virus
47,f	0.92	50	chronic fatigue syndrome
77.m	1.22	33	myelodysplastic syndrome, fatigue, anemia general
56,f	0.80	56	candiadiasis, allergic Rhinitis/Hay fever

Figure 1. Serum fluorescence emission for 40 normal subjects (mean ± 1SD) and for three illustrative patients with cancer (excitation wavelength 315 nm).
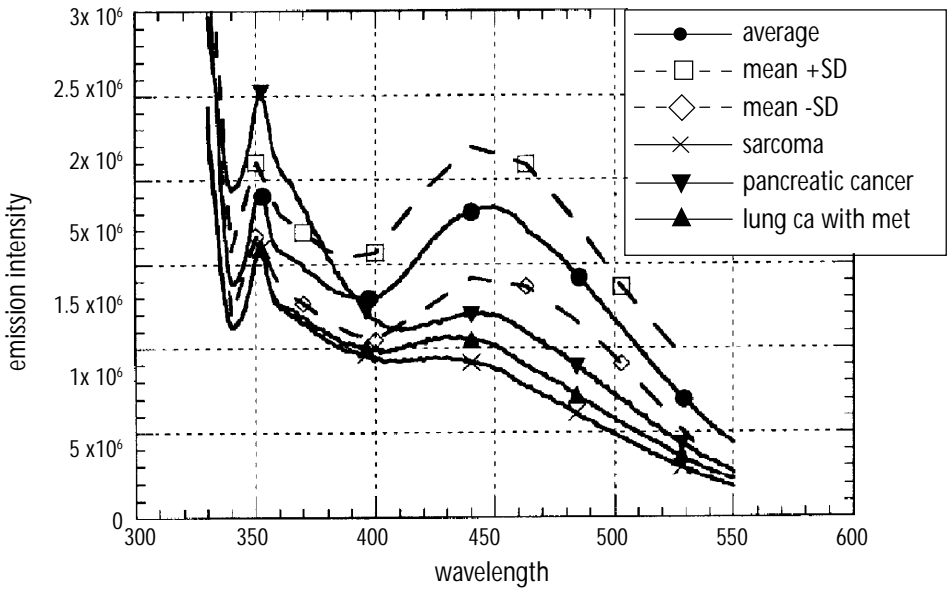

Figure 2. Distribution of serum emission intensities at 450nm for patients with cancer and for healthy volunteers.
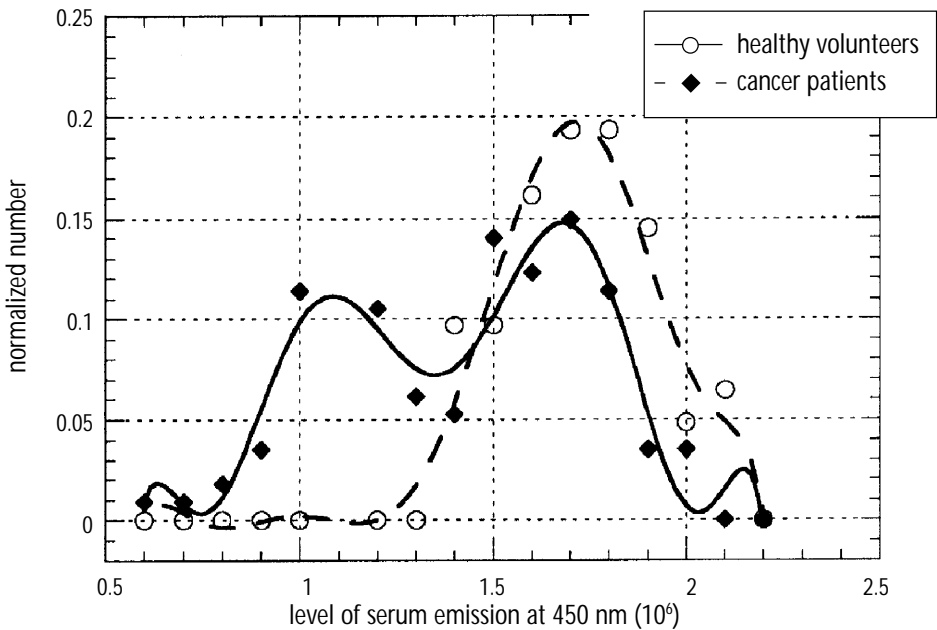
Table 2a. Results of measurements of the serum emission at 450nm and level of NAD(P)H emission for patients with cancer.

Age, sex	Intensity of emission (10^6 cps)	Decrease from average (%)	Equivalent of NADH concentration (µM)	Cancer type
68,m	1.53	16	9.52	prostate
64,m	1.62	11	10.07	lung
42,f	1.41	23	8.74	breast
62,m	1.55	15	9.70	colon
73,f	1.51	17	9.40	leukemia w/o remission
51,m	1.17	36	7.36	lung
55,f	1.19	35	7.48	chronic lymphocytic leukemia (CLL)
45,f	1.83	0	11.33	breast
62,m	1.43	22	8.92	lung
67,m	1.61	12	10.01	skin
67,f	0.93	49	5.92	lung, bone, brain
64,m	0.93	49	5.92	prostate
47,m	1.17	36	7.36	pancreas
80,m	0.97	47	6.16	esophagus, lung
74,f	0.8	56	5.13	pancreas, liver
57,f	1.61	12	10.01	breast, cancer neoplasm
39,m	0.76	58	4.89	stomach
54,m	1.49	19	9.28	bladder
46,f	1.48	19	9.22	breast
45,f	1.58	14	9.83	breast
47,f	1.53	16	9.52	breast
49,f	2.0	-9	12.35	breast
79,m	1.17	36	7.36	cancer neoplasm, lung
39,f	0.87	52	5.56	cancer neoplasm
59,m	1.72	6	10.67	colon
55,f	1.5	18	9.34	breast, cancer neoplasm
49,f	0.94	49	5.98	cancer neoplasm
45,f	1.5	18	9.34	breast, cancer neoplasm
52,m	1.59	13	9.89	renal cell carcinoma, lung
72,m	1.59	13	9.89	prostate
61,f	1.69	8	10.49	prostate
73,f	1.28	30	8.02	colon
67,m	1.4	23	8.74	prostate, lymes disease
67,m	1.44	21	8.98	prostate
56,f	1.68	8	10.43	breast
71,m	1.99	-9	12.29	pancreas
55,m	1.92	-5	11.87	prostate
51,m	0.86	53	5.50	pancreas
48,m	0.99	46	6.30	pancreas
78,m	1.69	8	10.49	breast
47,m	0.98	46	6.22	pancreas
50,f	1.82	0	11.27	breast
47,f	1.19	35	7.48	ovarian, uterus, cervix
53,f	1.69	7	10.52	bladder
63,m	1.49	19	9.28	colon
49,f	1.79	2	11.14	breast
72,m	1.45	20	9.09	liver
71,m	1.57	14	9.77	prostate
47,m	1.33	27	8.32	sarcoma
32,f	1.19	35	7.48	ovarian, uterus, curvix
62,m	1.34	27	8.38	cancer neoplasm
38,f	0.85	53	5.48	breast
42,f	1.18	35	7.47	breast

Table 2b. Results of measurements of the serum emission at 450nm and level of NAD(P)H emission for patients with cancer.

Age, sex	Intensity of emission (10⁶ cps)	Decrease from average (%)	Equivalent of NADH concentration (µM)	Cancer type
50,f	1.61	12	10.05	breast
45,f	1.55	15	9.65	breast
65,f	1.61	12	10.05	breast
50,f	1.78	2.62	11.04	breast
75,f	1.8	11	11.24	breast, multiple myeloma
80,f	1.68	8	10.44	breast
80,f	1.65	10	10.25	breast
45,f	1.48	19	9.25	breast, bone metastasis
50,f	1.46	20	9.15	breast, bone metastasis
79,m	1.15	37	7.27	chronic lymphocytic leukemia
63,f	1.41	22	8.86	CLL
73,f	1.48	19	9.25	leukemia
30,f	1.68	8	10.44	CLL
67,f	0.89	51	5.68	lung with metastasis
73,m	1.28	30	8.06	Lung
51,m	1.25	31	7.86	lung with metastasis
66,m	1.15	37	7.27	lung
51,m	1.25	31	7.86	lung
53,m	1.58	13	9.85	prostate
68,m	1.45	21	9.06	prostate
55,m	1.45	21	9.06	prostate
69,m	1.65	10	10.25	prostate
66,m	1.74	4	10.84	prostate
69,m	0.92	49	5.88	prostate
75,m	1.28	30	8.06	prostate
68,m	1.78	3	11.04	prostate
70,m	1.32	28	8.26	prostate
73,m	1.15	37	7.27	prostate
62,m	1.78	3	11.04	prostate
56,m	1.28	30	8.06	prostate
74,m	1.71	6	10.64	bladder, leukemia
46,m	0.99	46	6.28	hepatitis
86,m	1.41	22	8.86	cancer neoplasm
65,f	1.58	13	9.85	ovarian
54,m	1.8	4 0	11.44	Bladder
47,f	0.99	46	6.28	CLL
50,m	1.71	6	10.64	marginal cell leukemia
39,f	0.92	49	5.88	sarcoma
74,f	0.69	62	4.49	pancreases
73,f	1.25	31	7.86	colon
54,f	1.08	40	6.87	ovarian
59,m	1.56	14	9.75	multiple myeloma
75,m	1.98	-8	12.25	malignant lymphoma
51,f	1.55	15	9.65	liver cyst
47,f	1.15	37	7.27	ovarian
48,f	0.99	46	6.28	breast
62,f	1.48	19	9.25	Hepatitis C,
73,m	1.71	6	10.61	ovarian
63,m	0.99	46	6.28	pancreas
75,f	1.74	4	10.84	lymphoma malignant
70,f	1.64	10	10.84	lymphoma malignant
42,f	1.38	24	8.66	breast, liver
74,f	1.65	10	10.25	leukemia w/o remission

Sources of Emission

For identifying the different peaks in the serum emission spectrum derived from healthy volunteers and estimating the effect of different fluorescence components (proteins and coenzymes) on the native serum fluorescence, measurements of different fractions of fluorescent serum biomolecules were performed. Others have shown that fluorescence of native serum can be attributed to a variety of molecules such as tryptophan, tyrosine, phenylalanine, NAD(P)H, pyridoxal phosphate, bilirubin, kynurinine, flavin-adenine dinucleotide (FAD). The components used in our analysis were: nicotinamide adenine dinucleotide (reduced form) (NADH), 3-hydroxyanthranilic acid, 4-pyridoxic acid, pyridoxal-5-phosphate, L-tryptophan, kynurinine, nicotinamide and nicotinic acid (ordered from Sigma). The measured emission parameters for these fractions (excitation wavelength 315 nm) are listed in **Table 3** and presented at **Figure 4**. (p. 17)

As shown in Figure 4, the emission curves for the different fractions occurred at various wavelengths over the spectral region of native serum. The estimation of the contribution of several components in serum emission was performed by comparison the literature values for serum concentration of pyridoxal-5-phosphate (17 ±7 ng/dl), nicotinamide (340 nM), nicotinic acid (80 nM), kynurenine (<0.48 μM/dl) and analysis demonstrated low contribution of these fractions in total emission in analyzed range. Comparison of the emission of these fractions proved that there was no significant influence at the total native serum emission.

To analyze the contribution of the other fractions to the emission of serum, the mathematical programming tool, Matlab, was used to solve a series of linear equations using the following equation:

$$\sum_{1}^{5} Serum\ (\lambda i)= A i C_{NADH}(\lambda i)+A_2 C_{P\text{-}5\text{-}P}(\lambda i)+A_3 C_{4\text{-}p\text{-}acid}(\lambda i)+A_4 C_{3\text{-}h\text{-}acid}(\lambda i)+A_5 C_{trp}(\lambda i)$$

The intensities of emission at a specific

Figure 3. Ratio of the peak intensity at 450nm to valley at 400 nm for patients with cancer and for healthy volunteers.
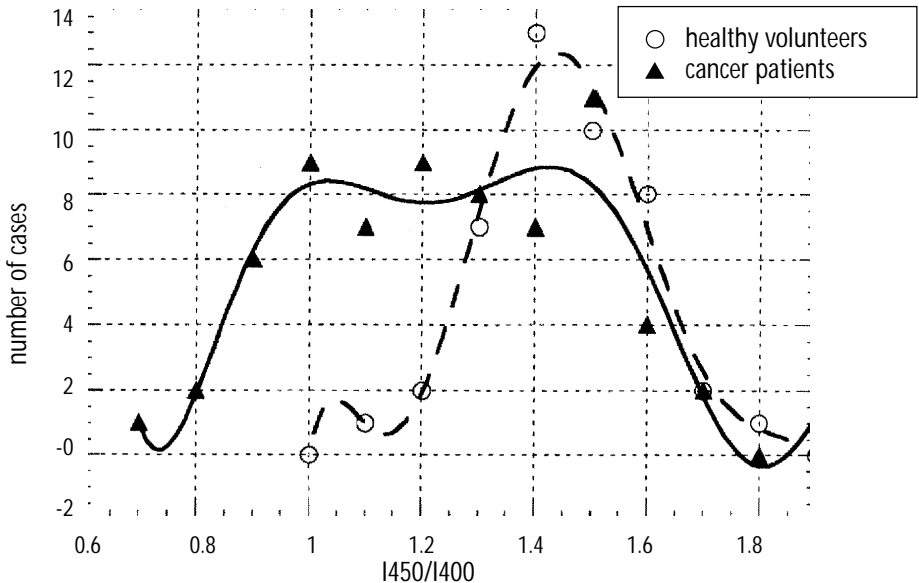
Table 3. Position of emission peaks for different excitation wavelengths for serum and different fractions of serum.

Emission of serum fractions and native serum emission	Excitation wavelength (nm)	Maximum of emmision (nm)
serum	315	450
	360	450
	380	453
	390	457
	400	465
	420	492
	450	520
3-Hydroxy-anthranilic acid	340	430
nicotinamide	315-360	386
l-kynurenine	315-360	488
nicotinic acid	315-360	416
NAD(P)H	340	460
Pyridoxal-5-phosphate	315-360	430
4-pyridoxic acid	340	431
tryptophan	315	365

Figure 4. The illustration of fluourescent emission for several fractions of serum.
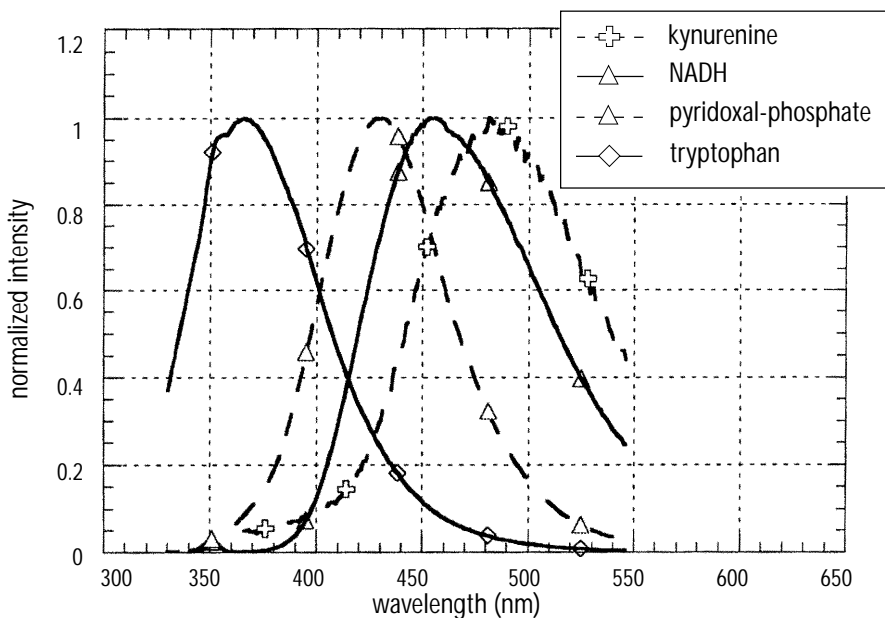
wavelength (li) for different wavelengths were calculated by Matlab program.

The five different components with adjustable coefficients An, were summed to give the experimental emission intensity of the native serum at a specific wavelength. The curve fitting results indicated that the values for the coefficients of 3-hydroxyanthranilic acid, pyridoxal-5-phosphate and 4-pyridoxic acid were small and the influence of emission from these fractions could be neglected. The calculated curves for emission from other fractions (NADH and tryptophan) and their sum are shown in **Figure 5.** (below) As noted in Figure 5, the calculated emission curve is in good agreement with the native fluorescence curve (average normal on graphs) and, hence, it may be described by emission of proteins and NADH in the 330 - 600 nm range.

Correlations Between Level of Serum Emission at 450nm and Laboratory Values for Patients with Cancer

For our cancer patients, there were available several laboratory measurements made on the same blood sample that was used for fluorescence emission data. These included: the complete blood counts, hormone T3, anti-Candida antibodies in blood (IgG, IgM, IgA), hemoglobin, hematocrit, MCV, glutathione in RBC, pyrroles in urine, Coenzyme Q10 and tumor markers: carcinoembrionic antigen (CEA) and prostate specific antigen (PSA).

We examined the data for possible correlations between these laboratory data and serum emission. The data presented in **Table 4** (p.19) demonstrate the coefficients of correlations calculated between level of serum emission and results of clinical tests.

For the collective data from all cancer

Figure 5. Comparison of the serum emission fractions with the most significant contribution and calculated sum of emission from these fractions with native fluorescence emission of serum.
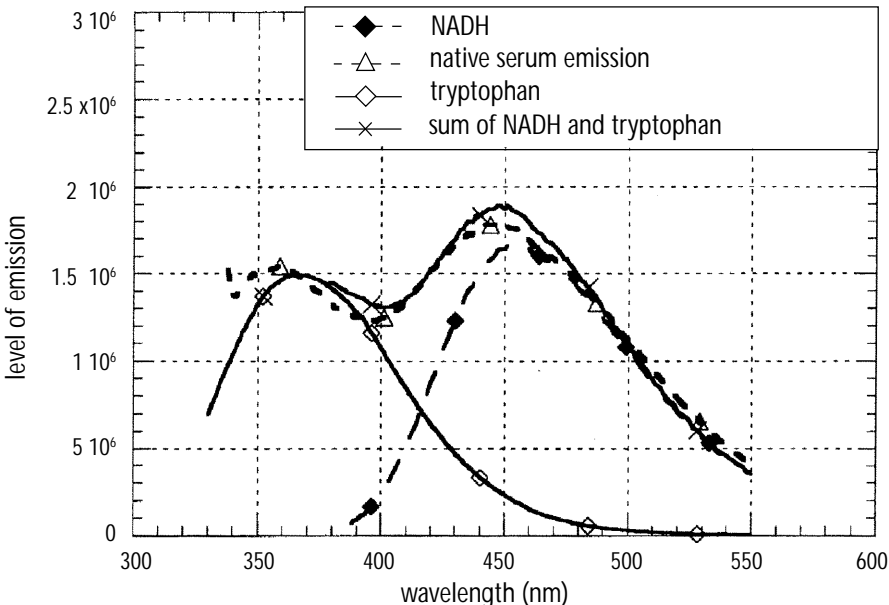
Table 4. Significant correlations (p<0.05) between level of serum emission at 450 nm and laboratory values for patients with cancer.

	Number of analyzed cases	RBC count	Level of hemoglobin	Anti-Candida antibody (IgG)	Glutathione in RBC	Pyrroles in urine	CEA	Coenzyme Q10
Cancer patients	90			-0.54 p<0.01	-0.63 p<0.01		-0.53 p<0.05	0.44 p<0.01
Breast cancer	28		0.54 p<0.05	-0.71 p<0.01				
Pancreas and liver cancer	13	0.71 p<0.05				-0.59 p<0.05		
Colon cancer	8	0.9 p<0.01	0.93 P<0.01	-0.83 p<0.05			-0.97 p<0.01	0.63 P<0.05
Lung cancer	15				-0.46	-0.53		

patients there were significant correlations between level of serum emission and level of glutathione in RBC (R = -0.63. p<0.01), level of tumor marker CEA (r = -0.53, p<0.05) and level of anti-Candida antibody (IgG) (R=-0.54, p<0.01). Correlation graphs are presented at **Figures 6,7.** (p.20)

Correlation between decreased level of emission and tumor marker CEA demonstrated that for all patients with the level of CEA greater than 10 ng/ml the level of serum emission (emission of NAD(P)H) was lower than average normal value by more than 20%.

For several groups of cancer patients there were also additional correlation of serum emission with values of RBC. Comparison of the red blood cell count, level of hemoglobin and hematocrit demonstrated correlation with level of serum emission for patients with breast cancer, cancer of the pancreas and liver, and colon cancer (**Table 4**, above). For this group of patients, there was the higher number of

cases when tests of blood count showed the abnormal level. Clinical tests for patients with prostate, lymphoma, lung and ovarian cancer mostly demonstrated changed level of serum emission and normal level of cell count or other parameters of RBC and WBC. Correlations between red blood cell count, level of hemoglobin and level of serum emission at 450nm for patients with breast cancer, pancreatic cancer, liver cancer and colon cancer are presented in **Figures 8**, (p.21) and **Figure 9**, (p.22).

We also compared the level of serum emission at 450nm with level of coenzyme Q10 in serum. Coenzyme Q10 is one of the parts of complex series of reaction that occur within mitochondria. The function of Q10 is ultimately linked to the generation of energy within the cells. The reversible oxidation and reduction of coenzyme Q10 is the basis for its function as a carrier of electron between flavoproteins and

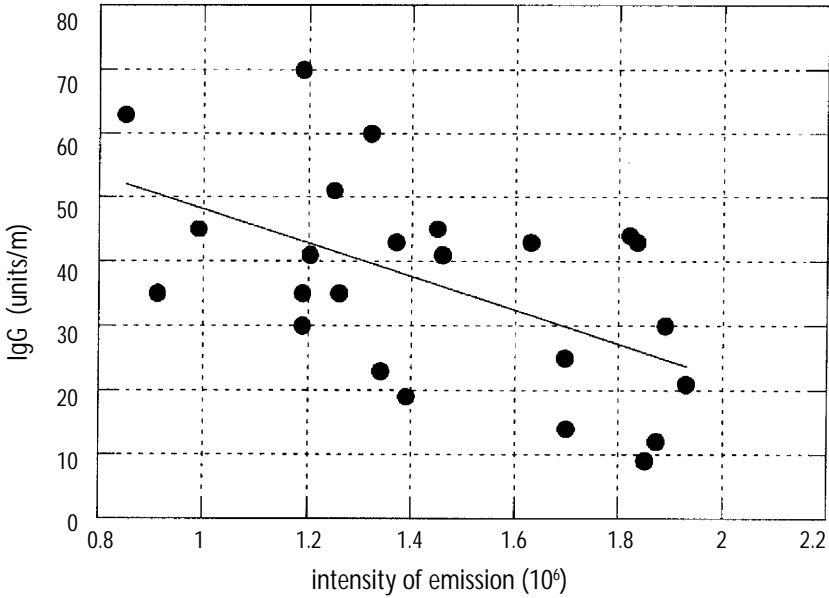**Figure 6.** Correlation between level of serum emission at 450nm and level of anti-Candida antibody IgG in serum.

Figure 7. Correlation between level of serum emission and level of glutathione in RBCs.
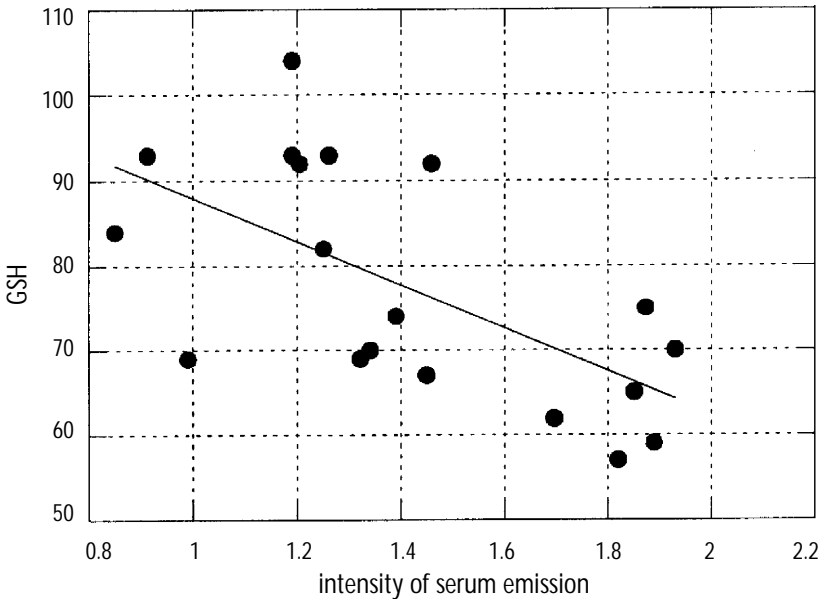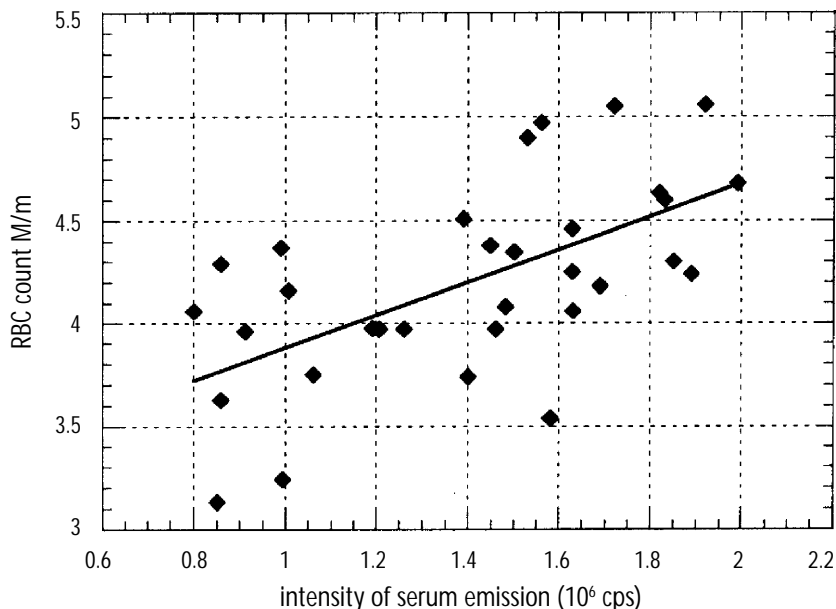
Figure 8. Correlation of the level of serum emission with red blood cells' count for patients with breast cancer, liver cancer, cancer pancreas and colon cancer.

cytochromes. Coenzyme Q10 is essential for ATP production and for bioenergetics. In comparison with other respiratory carriers in the inner mitochondrial membrane, the content of Q10 exceeds the other redox components by about tenfold. Deficiencies in CoQ10 are implicated in heart disease, infection, cancer and AIDS. The low level of bioenergetics may be due to exhaustion of coenzyme Q10 store.

We analyzed the level of coenzyme Q10 and the level of NAD(P)H in serum to compare the bioenergetic role of these biomolecules. The data presented in Table 4 demonstrated correlation of the decreased level of serum emission (level of NAD(P)H) with decreased level of coenzyme Q10 (R = 0.44, P<0.01).

Discussion

We analyzed the possible reasons for the change in serum emission for these groups of patients. The main source of coenzymes in serum may be the storage of the NAD in serum due to conversion of tryptophan and nicotinic acid, the process which is controlled by the liver in a hormone-sensitive manner,[14] leakage of these biomolecules after the breakdown of cells circulating in blood (red blood cells, white blood cells) and after breakdown of tissue cells. Tissue level of NAD+ is regulated by the concentration of extracellular nicotinamide.

The changed level of energy production and changed level of metabolism in cells during the development of the pathological conditions may be associated with abnormalities of oxido-reductase enzymes. Changes in the NADH level, NAD redox potential (ratio of NADH to NAD+ NADH) and decrease the NAD redox potential and NADH level in cells was found for patients with different pathological conditions and correlated with the decreased level of enzymes.[15-18]

Figure 9. Correlation of the level of serum emission with level of hemoglobin in RBC for patients with breast cancer, liver cancer, cancer pancreas and colon cancer.
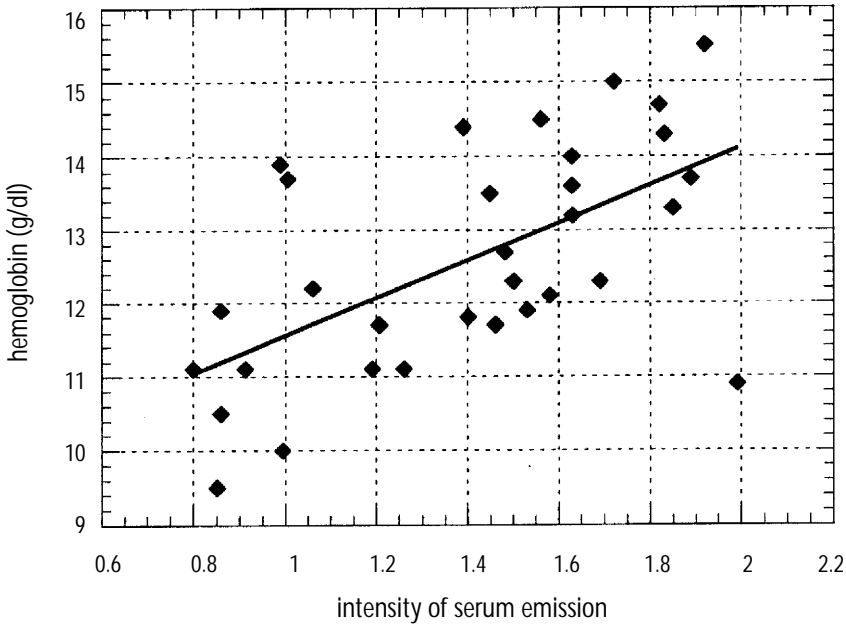
During tumor development the interlocking coordination is defective in cells. The glycolysis, pyruvate oxidation, the citric cycle and oxidative phosphorylation are regulated by the relative concentrations of ATP, ADP and NADH and changes in metabolic rate may influence the concentration of these molecules. Also at least some cancer cells undergo a decrease in T3 receptors, a decrease in response to T3 receptors and a decrease in production of T3 and T4 receptors. Additional evidence that cancer patients have a below normal levels of serum NADH comes from the fluorescence spectroscopy measurements of virus-transformed cells.[7] The significant difference between normal and transformed cells appeared to involve intracellular NADH metabolism, which resulted in a much lower fraction of free NADH.

Also concentration of adenine (ATP, ADP, AMP) and pyridine (NAD(P), NAD(P)H) nucleotides in the erythrocytes may be changed for patients with cancer.[18] Possibly, if a level of ATP is lower in the erythrocytes of the subjects affected by tumors, it also may be accompanied by modifications in the concentrations of other energetic molecules and lower level of NAD(P)H in cells and lower level of these coenzymes in serum.

As the main fraction of NAD(P)H in serum is due to leakage from cells, all these observed effects may be the reason for decreased level of serum emission at 450 nm.

In our measurements serum fluorescence intensity at 450nm (NAD(P)H emission) was lower for many cancer patients than the intensity of emission at the same wavelength for the healthy volunteers.

As the result of our analysis the correlation was found between levels of GSH and serum levels of NAD(P)H, we propose that the decreased level of energy metabolism may be due to oxidative stress and damage of cells and, in particular red blood cells, by reactive oxygen metabolites. The oxidative stress is considered to be involved in the pathophysiology of cancer. Oxidative damage of RBC and exposure of cells to oxidants causes multiple intracellular alterations, including depletion ATP and oxidation of NADH, reduction glutathione (GSH) and lipids. Peroxidation of unsaturated fatty acids makes them more hydrophilic and alters the structure of the membrane and changes normal membrane function. For example, red cells may be so damaged by reactive oxygen species (ROS) and peroxidation that they more rapidly degrade resulting in anemia. ROS or peroxides further may cause oxidation of iron in hemoglobin, resulting in the formation of methemoglobin, a molecule incapable of transporting oxygen. Increased level of methemoglobin and decreased level of oxygen delivered to cells cause a decrease in the level of energy metabolism and fatigue in cancer patients. Correlation of the level of NAD(P)H in serum with tumor marker CEA indicates that the level of energy metabolism decreases during the development of disease. Also correlation of the GSH level with level of NAD(P)H demonstrated that depletion of the NAD(P)H may be caused by conditions of increased demand for reducing GSSG. GSH is one of the components of the defense system. Many radicals and nonradicals reactions in cells lead to oxidation of glutathione to form GSSG. The regeneration reaction of the reduction of GSSG to GSH catalyzed by GSSG reductase uses NADPH as reducing equivalent.

The reactive oxygen metabolites released by tumor cells or generating as the result of toxic depression, nutritional inadequacies, results of chemotherapy or other treatments may also damage white blood cells and cause overgrowth of yeast, fungi or bacteria during the pathological conditions. This conclusion may be supported by found correlation between level of anti-Candida antibody IgG and level of NAD(P)H in serum (r=-0.54, p<0.01).

Cancer patients claim that fatigue is the most debilitating side effect of cancer and its treatment affecting their ability to cope with cancer therapy. Accurate diagnosis of the fatigue and anemia are very important for proper treatment of cancer patients. As the fatigue-like pain is not easy to measure, our method may be useful for measuring this parameter for cancer patients.

Conclusion

A change in energy metabolism is commonly observed in various diseases. Measurements of the level of NAD(P)H and level of energy metabolism for patients with cancer is important because it allows measuring fatigue levels. Fatigue due to oxidative stress and anemia leads to a decrease in the quality of life since oxygen is like energy. High level of oxidative stress and decreased level of oxygen carrying capacity of RBCs may lead to increased tumor hypoxia, increased drug resistance, increased tumor angiogenesis and decreased survival of patients. Decreased level of energy metabolism reduces survival times in patients with various malignancies. Thus, a method to routinely determine ones energy level would be helpful for risk assessment.

A procedure of serial sampling would also be useful to monitor the metabolic state of patients under intensive care during critical illnesses. A non-invasive methodology has been designed in this report to estimate the level of energy metabolism by measuring the fluorescence of reduced nicotinamide adenine dinucleotide. Our analysis during two years of the level of serum emission for patients with different metabolic disorders showed characteristic changes in serum emission for patients with cancer, CFS and other groups of chronic diseases.

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