Multielement Correlation Analysis of Major-to-Trace Elements in Human Blood Serum for Medical Diagnosis as Studied by ICP-AES and ICP-MS

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The major-to-trace elements in the human blood serum samples, collected from the healthy volunteers as well as from the patients of specific diseases, were determined by ICP-AES (inductively coupled plasma atomic emission spectrometry) and ICP-MS (inductively coupled plasma mass spectrometry) after acid digestion. The whole blood samples were collected with the silicone-coated syringes and centrifuged at 3000 rpm to obtain the serum samples. Then, the blood serum samples were digested with nitric acid on an aluminum bath. Finally, the residue of blood serum was dissolved with 0.1 M nitric acid to subject to the analyses by ICP-AES and ICP-MS. The analytical results were summarized as the radar charts, which indicated the relative concentrations of 14 elements in order to perform the multielement correlation analyses for medical diagnosis. Such multielement radar charts for specific disease patients were compared to those for the healthy volunteers. It was found that the concentrations of P and Cu in sera collected from healthy females were higher than those from healthy males, while those of Fe, Zn, Mo and Ag in sera from the former were lower than those from the latter. Furthermore, the significant increases in Cu, Se and Mo were clearly observed for non-Hodgkin's lymphoma (blood cancer) patients.

(Received on August 8, 2001; Accepted on September 13, 2001)

Introduction

It is well known that various elements are present in our human body in the wide concentration ranges, and many of them play important roles in blood and organs.[1] For example, various enzymes containing Zn play essential roles in physiological functions and biological synthesis. In addition, Fe is responsible for the transport of molecular oxygen in the blood, and Cu is engaged specifically with redox reactions in human body. In recent years, it has been also reported that various elements inter-dependently work through the mutual interactions in human body.[2] Therefore, further information about the distributions and kinetic behaviors of major-to-trace elements in human body is really required for elucidation of their biological roles and functions on the multielement basis. Human blood serum is one of the biological fluids most frequently used in clinical analysis for medical diagnosis, because it is easily collected from man. In the present study, thus, the blood serum samples were used for multielement correlation analysis of major-to-trace elements.

The concentrations of the elements in blood serum are influenced by physical conditions, pathological conditions, sexes, ages, meals and so on. Therefore, it is the most difficult task how to get the background concentrations of the elements in blood serum. In the present experiment, the blood serum samples donated by the 92 healthy volunteers were analyzed to obtain the background concentration levels of major-to-trace element in human blood serum. In addition, the blood serum samples collected from 32 non-Hodgkin's lymphoma (NHL) patients were also analyzed for medical diagnosis. Then, comparison between males and females as well as between the healthy volunteers and NHL patients was made to elucidate differences of the concentration distributions of the elements in blood serum. The main purpose of the present research, thus, is that some indices for health and disease would be obtained from multielement correlation analyses of the elements in serum.

Experimental

Instrumentation

An ICP-AES instrument of Model Plasma AtomComp MK II from Jarrell Ash (Franklin, MA, USA), which consisted of a direct-reading polychromator of Paschen-Runge mounting with 39 channel detectors (photomultipliers), was used for the determination of Na, K, P, Ca, Mg, Fe, Cu and Zn in the serum samples. A cross-flow type nebulizer for pneumatic nebulization was employed in the ICP-AES instrument. The ICP-MS instrument of Model SPQ 8000A (Seiko instruments, Chiba, Japan), equipped with a quadrupole-type mass spectrometer, was also used for the determination of Se, Rb, Sr, Mo, Cs and Ag in the serum samples.

Table 1 Analytical results for the elements in blood sera collected from healthy volunteers and NHL patients

Element[a)]	Concentration[b)] / μg g^{-1}					
	Males		Females		Total	
	Healthy (*n*=45)	NHL[c)] (*n*=12)	Healthy (*n*=47)	NHL[c)] (*n*=20)	Healthy (*n*=92)	NHL[c)] (*n*=32)
Na†	3080	2940	3170	2930	3130	2940
K†	149	148	152	151	151	150
P†	117	118	122	123	120	121
Ca†	93.3	84.1	93.0	84.0	93.1	84.0
Mg†	17.8	18.9	17.3	18.2	17.5	18.5
Fe†	1.46	1.54	0.94	1.01	1.20	1.23
Cu†	0.73	1.00	0.77	1.11	0.75	1.06
Zn†	0.71	0.65	0.60	0.64	0.65	0.64
Se	0.164	0.227	0.156	0.219	0.160	0.222
Rb	0.169	0.145	0.169	0.139	0.169	0.141
Sr	0.0324	0.0280	0.0339	0.0342	0.0332	0.0317
Mo	0.00162	0.00237	0.00118	0.00260	0.00140	0.00251
Cs	0.00066	0.00058	0.00066	0.00060	0.00066	0.00059
Ag	0.00018	0.00031	0.00021	0.00038	0.00020	0.00035

a) The elements with † were determined by ICP-AES and others by ICP-MS. **M**: Bio-essential element.

b) The mean values for volunteers and patients. The numbers of the samples are shown in the parentheses of the corresponding columns.

c) NHL: non-Hodgkin's lymphoma patients.

Chemicals

The multielement standard solutions for standardization of calibration curves were prepared from the commercially-available single-element standard solutions (1000 μg ml^{-1}) for atomic absorption spectrometry, purchased from Wako Pure Chemicals (Osaka, Japan). Hydrochloric acid used was of electronics industry grade, and nitric acid used was of Ultrapur grade, all of which were purchased from Kanto Chemicals (Tokyo, Japan). Pure water used throughout the present experiment was prepared by a Milli-Q water purification system (resistivity of 18 MΩ cm, Nihon Millipore Kogyo, Tokyo, Japan).

The centrifuge tubes were cleaned before use as followed: a rubber stopper of the tube was removed and its mark was rubbed out carefully, and them the tube was soaked in 2 M HCl for 2 days. The tube soaked was rinsed with pure water and dried before use. The polypropylene cap was washed in the same manner. For storage of the serum samples, 15 ml centrifuge tube (polypropylene tube: CORNING, USA) was used.[3]

Blood serum samples

Human blood samples were collected from healthy volunteers and NHL patients by a 20 ml polypropylene syringe (TERUMO, Tokyo, Japan) equipped with a silicone-coated venipuncture needle (22Gx$^{1}/_{4}$, TERUMO), and they were transferred to the centrifugation tubes, venoject tubes (pain, silicone-coated, TERUMO). After clotting, the blood samples were centrifuged at 3000 rpm for 20 min. The supernatants were used as the serum samples.

NHL (non-Hodgkin's lymphoma) is the disease that lymphocyte is transformed to malignant alteration. In Japan, it is considered in medicine that most of patients with malignant lymphoma are NHL patients.

Digestion procedure of human blood serum

The serum samples analyzed in the present experiment were digested as follows. The serum sample (*ca.* 0.5 g) was taken in a PFA tube (6 ml in volume), which was cleaned by boiling in the diluted (2-fold) *aqua regia* for 8 h, and the PTFE ball was put as the cover cap on the top of the tube. After adding 1 ml of *conc.* HNO_3, the serum sample was heated on an aluminum heating-block at 110 ˚C for 5 h. After removing the PTFE ball, the sample was further heated almost to dryness at 200 ˚C. Finally, the residue was dissolved with 5 ml of 0.1 M HNO_3, which contained the internal standard elements (Ge, Rh and Re; 10 ng ml^{-1} each). Thus obtained serum solutions were subjected as the final analysis solution to the ICP-AES and ICP-MS measurements. The digestion procedure described above was carried out in a clean draft (Yamato Science, Tokyo, Japan).

Results and Discussion

The analytical results for human blood serum are summarized in Table 1, where the observed values for 92 healthy volunteers and 32 NHL patients are shown separately in the case of males, females and the total. In order to examine the concentration distributions and their variations, the relative concentrations of 14 major-to-trace elements and albumin to their averaged concentrations obtained for healthy volunteers and patients[4], were illustrated as the radar charts in Figs. 1-4

Sexual differences of the elemental concentrations in blood serum from males and females

The analytical results obtained for 45 healthy males and 47 healthy females are summarized in Figs. 1 and 2, respectively. As for Na, K, P, Ca, Mg, Cu, Zn, Se and albumin, in general, their concentrations in the serum samples from healthy volunteers were distributed in rather narrow ranges, which were in the normal distributions. These facts may indicate that homeostasis for these elements are kept in human blood serum. On the other hand, the concentrations of Fe, Sr, Mo, Cs and Ag showed significantly wide distributions in both males and females, and they were in some logarithmic-normal distributions. Iron is known to be rapidly exchanged between serum and erythrocytes which is in the reduction or anaerobic conditions, although the biological functions of Mo and Ag in blood have not been well known yet.

The concentrations of P and Cu in sera collected from females are higher than those from males. Estrogen, which is a sex hormone, receives some influences of Cu and P. In fact,

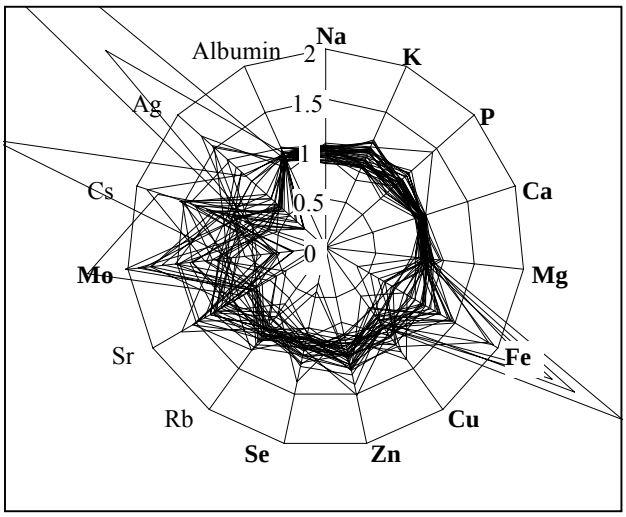

Fig. 1 Radar chart for the concentration distributions of the elements and albumin in blood sera collected from healthy male volunteers. The elements written with bold letters indicate bio-essential elements.

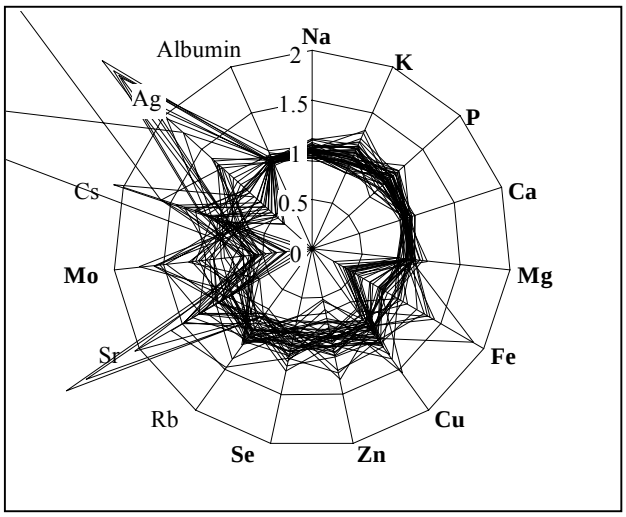

Fig. 2 Radar chart for the concentration distributions of the elements and albumin in blood sera collected from healthy females volunteers. The elements written with bold letters indicate bio-essential elements.

the concentrations of Cu and P in female serum are in fairly good correlation with each other. On the contrary, the concentrations of Fe, Zn, Mo and Ag in sera from healthy females were lower than those from males. Females bleed by period, which results in the lower concentration of Fe existing much in the blood. Sodium, K, Ca, Mg, Se, Rb, Sr and Cs were almost at the same concentration levels in males and females.

Comparison of the elemental concentrations between blood sera collected from healthy volunteers and non-Hodgkin's lymphoma patients

The analytical results for 12 NHL male patients and 20 NHL female patients were summarized in Figs. 3 and 4, respectively, where the data obtained from all patients are plotted as the relative concentration of each element. The concentrations of Cu, Se, Mo and Ag in sera collected from NHL patients of both male and female were significantly higher than those from healthy volunteers. The concentration increase of Cu in serum may be explained by suppression of from tumor cell growth due to Cu. Another possibility is production of free radicals by excess Cu, which also results in destruction of DNA for cancer cell growth.[5-10] The increased Se, which is contained as glutathioneperoxitase in blood serum, may eliminate active oxygen and suppress inflammation. It is also said that Se has an immunological enhancement effect. The concentrations of Cu and Se in sera of healthy volunteers were in a positive correlation, while those of Cu and Se in sera of NHL patients were in a negative correlation. It should be noted here that the concentrations of Cu and Se in sera collected from NHL patients showed a negative correlation, even though their concentrations of Cu and Se were increased compared to those from healthy volunteers.

The concentrations of Zn in sera collected from NHL male

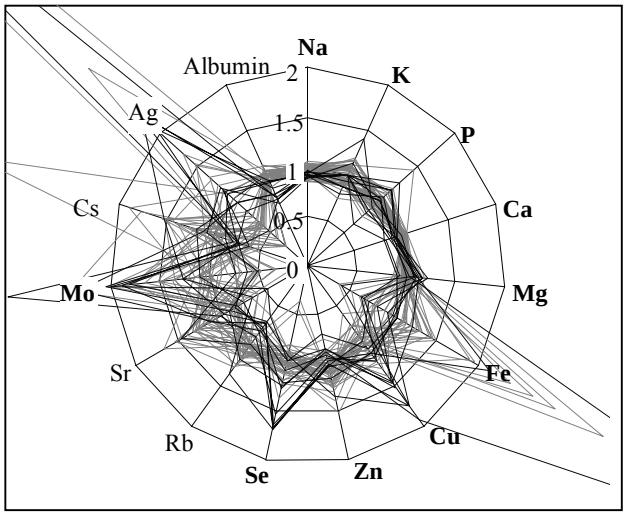

Fig. 3 Radar chart for the concentration distributions of the elements and albumin in blood serum collected NHL male patients (——), where the distributions for healthy males are also shown by gray lines. The elements written with bold letters indicate essential elements.

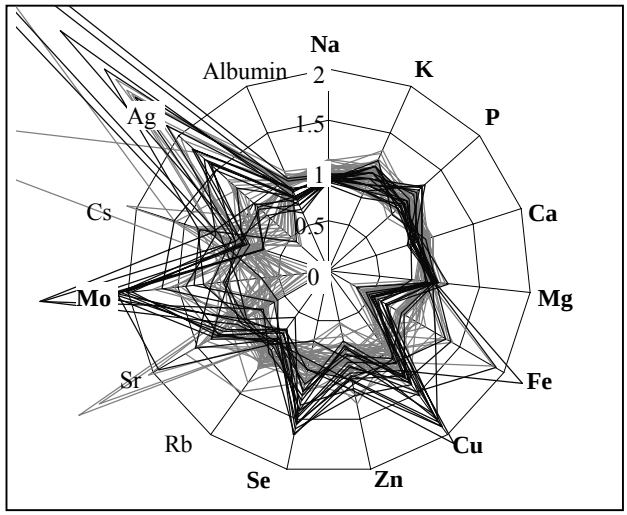

Fig. 4 Radar chart for concentration distributions of the elements and albumin in blood serum collected from and NHL female patients (——), where the distributions for healthy males are also shown by gray lines. The elements written with bold letters indicate essential elements.

patients were decreased, compared to those from healthy males, while the concentrations of Zn in sera from NHL female patients were higher than those from healthy females. It should be noted that Zn is contained in DNA and RNA polymerases as well as various enzymes for proteins synthesis. Thus, the decrease of Zn in serum causes Zn deficiency, which may result in the increase of cancer cells deterioration of immunological functions.

Conclusion

The multielement determination of major-to-trace elements in human blood sera collected from healthy volunteers and NHL (non-Hodgkin's lymphoma) patients were carried out for medical diagnosis. As a result, the following conclusions have been drawn from the present experiment. The concentrations of P and Cu in sera collected from healthy females were higher than those from healthy males, while the concentrations of Fe, Zn, Mo and Ag in sera from healthy females were lower than those from males. Other elements determined here were almost at the same concentration levels in healthy males and females. The concentrations of Cu, Se, Mo and Ag in sera collected from NHL patients were significantly increased, compared to those from healthy volunteers. Multielement correlation analysis of the elements in blood serum for healthy volunteers and NHL patients is useful for diagnosis of health conditions, and thus, it is expected, in near future, that multielement correlation diagnosis of human blood serum would be applied to the chemotherapic treatments for the patients with various infections.

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