

Effect of Chemotherapy on Zn, Fe, Mg, Pb, Ca and Se in the Serum

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Abstract

Trace element concentrations in blood serum are very important in various carcinomas. However, the literature lacks in studies of the relationship between the trace element concentrations and cancer types. The present study analyzes blood serums from 40 patients with throat, stomach and lung cancers by inductively coupled plasma optical emission spectrometry (ICP-OES) after low-volume microwave digestion. 7 elements were investigated: Se, Ca, Fe, K, Mg, Pb, and Zn. The elements were categorized into macro elements (Ca, Mg, K) and trace elements (Fe, Zn, Pb, Se) for human blood. The mixture of $\text{HClO}_4:\text{HNO}_3:\text{H}_2\text{SO}_4$ was selected for analyzing the trace elements in serums taken from cancer patients. The digested blood serums of pre-chemotherapy and post-chemotherapy were analyzed to investigate the effect of chemotherapy on amounts of trace elements. Decreases were observed in amounts of the essential trace elements except Pb for post-chemotherapy by time, as biochemical reactions were catalyzed by many enzymes and proteins. Certain changes must be made in the scientific approach in cancer therapy. Such changes in treatment of the patient with cancer should include continuous treatment of each patient individually according to the results obtained.

Keywords: Chemotherapy; Trace elements; ICP-OES; Throat cancer; Stomach cancer; Lung cancer

Introduction

Recently, cancer cases have increased considerably in the last decades. The reasons for this increase is connected multi-dimensional and complex relations such as economic development and its effects on human life: the increase in the intake of unnatural foods, breathing polluted air, increased quantity of trace elements in drinking water. The trace elements are play important role in this dramatic increase in cancer cases and more and more factors are affecting the quantities of trace elements in human body. This caused a great deal of attention on the determination of trace elements [1-11]. The change in the balance of trace elements is the most important factor in this situation and any deviation from the optimum levels of them may have a drastic effect on biological processes. In addition, trace elements like Cu, Zn, Ca, Mn, Fe, etc have decisive functions in maintaining human health and are of great importance for the synthesis and structural stabilization of both proteins and nucleic acids. In this context the role of Se in preventing and ratio of Cu/Zn as an indicator for various cancers or tumors are widely investigated. However it is highly difficult to explain the relationship between trace elements and some diseases. Further studies are needed for the clarification of these relations. Blood is the most commonly used sample to identify the trace elements due to ease of sampling. In addition it is the medium of transport of trace elements to tumors and healthy organs. Therefore, whole blood, plasma, and serum are commonly used for determinations. Cancer is known as a genetic disease, but diet and environmental conditions have a significant effect on cancer incidents. For instance, 65-70% of all cancers are associated with the environmental conditions and only 2% is related to genetic factors. Moreover, some of the trace elements are important for human health and some are highly toxic. However an excessive intake any elements causes serious complications including cancer [1-4]. Trace elements in human body are inessential and essential for the growth of the organism due to their electrochemical and catalytic effects. They activate enzymatic reactions or inhibit *in vitro* reactions. Zn, Ca, Mg, Fe and Se are known as essential trace elements due to their catalytic functions for the formation of various essential compounds with proteins. Fe also catalyzes many redox reactions. However some of the trace elements catalyze the oxidative damage of biological macromolecules and generate free radicals [5]. Zn and Pb have been used to induce cancer. The metal ions can interact with nucleic acids to influence base pairing and conformation. Se has anticarcinogenic

properties, but its quantity in serum and whole blood or tissue is important. For instance, toxic level of the element causes chronic toxic hepatitis that looks like hyperplasia. It has anticarcinogenic effects by several mechanisms and protective action against chromosomal damage. A part from their role in antioxidant defense system, seleno proteins break downs in hydrogen peroxide and lipid hydroperoxides, which can damage cell membranes and disrupt cellular functions. Pb is a toxic metal and causes a serious hematological damage, brain damage, anemia, and kidney malfunctioning [12]. Zn concentration affects growth retardation, anorexia, delayed sexual maturation, anemia, mental retardation, impaired visual and immunological function, etc. Low levels of zinc cause some dysfunctions of the immune system [13]. Serum concentrations of Zn are modified in some cancers; variations of Zn concentrations have been observed in leukemia. There is an important difference in the role of Se and Zn in a number of cancer types. There is an inverse relationship with cancer and quantity of Se and Zn has a protective effect when it is present in sufficient quantities [14-16]. Pb has an adverse effect on Fe deficiency, and Fe deficiency increases the absorption of Pb [17]. The absorption of Pb is positively correlated by the lower presence of Ca [18]. Fe is an important part of hemoglobin and myoglobin. Mg^{+2} ions are used for activating all enzymes used for transferring phosphate from ATP to acceptor molecule or ADP. Therefore, 1/3 of total quantity of Mg is observed to be bounded to proteins. Chemotherapy used in cancer treatment often causes many side effects depending on the type of cancer, location, drugs and dose and general health. This is because it has an important function on active cell growth and propagation. The most effected cells are the ones in our blood, mouth, digestive system, and hair follicles which cause various side effects. The elimination of these side effects involves the use different medications. Chemotherapy also affects the quantity of trace elements which play important roles in a number

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of processes occurring in human body by activating or inhibiting enzymes. There is a little research on the quantity of trace elements in the blood pre-and post-chemotherapy periods. The dissolution of the blood samples in a suitable media is necessary to determine trace elements. Some dry and wet ashing procedures are available. Different acid mixtures such as $\text{HCl}:\text{HNO}_3$, $\text{HClO}_4:\text{HNO}_3$, $\text{HClO}_4:\text{HNO}_3:\text{HCl}$ and $\text{HClO}_4:\text{HNO}_3:\text{H}_2\text{SO}_4$ for Pb, Cu and Se determination can be used [19]. Especially, there may be substantial loss trace elements during the decomposition of organic substances. Se is much more volatile than trace metals like Cu and Pb. When acidic mixture does not contain oxidizing substance such as HNO_3 , partial losses of selenium as SeO , Cl_2 , SeO_2 , 2HCl , etc. has been observed [19-23]. Despite the existence of various analytical methods for the determination of trace elements, most of them are limited with a few trace elements including the ones in blood during the pre-chemotherapy and post-chemotherapy, and the last stage of patient life with cancer. Consequently, this paper investigates the correlation between the quantities of trace elements in serum and chemotherapy for different types of cancers. This study is expected to contribute to medicine as regards to chemotherapy process which is the most common mode of cancer treatment.

Experimental

Reagents and standard solutions

All acids used were of Arist and other reagents were of analytical grade purity. Standard working solutions were obtained from a multielement standard stock solution ICP Multi Element Standard Certipur VI (Merck, Darmstadt, Germany) after suitable dilution. Homemade triply distilled water was used for the preparation and dilution of solutions. Calibration curves were plotted from three replicates measurements and, in all cases, regression coefficients were higher than 0.999. Platinum was chosen because it contained negligible amounts of the metals of interest. The internal standard solution was added to all solutions automatically using an on-line addition (265.945 nm).

Instrumentation and analysis of trace elements

Concentrations of Zn, Fe, Mg, Pb, Ca, and Se in serum of patients were analyzed by ICP-OES using a Perkin-Elmer Optima 4300 DV spectrometer (Shelton, CT, USA), equipped with an AS-90 auto sampler, axial system, a high dynamic range detector and a cross-flow type nebulizer for pneumatic nebulization. The ICP-OES measurement conditions for these elements were optimized to achieve the maximum signal-to-background ratio. A line selection was performed to determine the elements analyzed in the study and detection limits were determined by using an appropriate blank solutions and aqueous standard solutions three times, and thus the LOD was calculated. The instrument was operated under suitable conditions. Different concentrations (0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 mg/L) of trace elements were used for calibration of standard graphs. To verify the assay accuracy and to maintain quality, the standard solutions were run for every 10-test sample.

Blood sample collection preparation and sample analysis

The blood samples collected from 40 volunteer patients in Ankara Oncology Training and Research Hospital to control the results of chemotherapy 0.5 ml serum of the bloods was used for the determination of the trace elements. 5 mL of concentrated HNO_3 was added and waited for 2 minutes. Then, each of the samples waited for 12 h at room temperature by adding 5 ml of concentrated HNO_3 plus approximately 5 drops of H_2O_2 . Afterwards, the resulting solutions

were heated to approximately 120°C . The digested residues were redissolved and filled in 5 mL flasks. To prepare blank solutions, the same amounts of chemicals used for sample digestion and performing the same dissolution stages were used. For the determination of recovery, a known amount of single element standards was added to the samples with known concentrations prior to wet digestion. One of the most difficult parts of this analysis is getting the sample introduction system clean enough to determine low levels of Zn, Fe, Mg, Pb, Ca, and Se. With the demountable torch cassette design, it is very easy to switch to a different sample introduction system. In addition, this design incorporates a short transfer line between the spray chamber and torch to minimize sample carry-over and contamination. The most difficult part of the analysis is sample introduction system to determine low concentrations of the trace elements. Demountable torch cassette design was used to minimize sample carry-over and contamination. Before the analysis, the nebulizer, spray chamber, injector adapter, injector, and torch were cleaned by soaking for 30 minutes in warm diluted nitric acid. This action provided a clean sample introduction system to measure these low-level analyses.

Results and Discussion

The analysis of heavy metals requires the elimination of the organic compounds. However the digestion of organic compound may result in loss of trace elements [24]. Since the volatility of Se is higher than Zn, Fe, Mg, Pb, and Ca it is necessary to use an oxidizing agent in order to obviate partial losses of selenium. The use of oxidizing acids in digestion may cause the conversion of Se(IV) to Se(VI). Therefore one must use high concentrations of HCl (4-7 mol/L) to avoid the back oxidation from Se(IV) to Se(VI) [24-29], since the only compound formed from Se(IV) is selenium hydride. In contrast, HCl concentration has to be higher than 2 mol/L for formation of Se(IV). Aydın observed good results from colorless clear serum solution for trace quantities of Se and the other elements. The researchers proposed that mixture of $\text{HClO}_4:\text{HNO}_3:\text{H}_2\text{SO}_4$ was suitable for the oxidation of Se and the other metals except high concentration of Pb in organic medium due to precipitation of PbSO_4 . Serum Trace Elements Profile in Lung, Throat, and Stomach Cancers. There is a vast number of papers on the role of trace elements in various cancer cases and the relation between types of cancer cases and amount of trace elements in human serums in the literature. Collecting the samples without contamination and analyzing them for trace elements are highly cumbersome. Trace elements in serums of lung cancer patients have been analyzed pre-and-post-chemotherapy periods. Tables 1-3 compare concentrations of essential trace elements in serums taken from patients with lung, throat and stomach cancers and show the effect of chemotherapy on amounts of trace elements. The elements are categorized into macro elements (Ca, Mg, K) and trace elements (Fe, Zn, Pb, Se). Differences in amount of trace elements were important between pre-chemotherapy (week 1) and post-chemotherapy (week 2-4), but quantities of the elements were low than that of patients without cancers except selenium (Tables 1-3). The source of the observed differences in four weeks for each patient and among patients may be the differences in eaten foods, previous treatments before chemotherapy, genetics of patients, and habit of tobacco, alcohol, dietary supplements consumption frequency of meat consumption. However, there were significant differences between levels of the elements compared to the elements in serum samples in cancer patients as compared to those without lung and throat cancers except selenium. Decreases in amounts of the elements except Pb were observed in the overall amount of some of essential trace elements for post-chemotherapy by time. The decreases in the amounts show a decrease in biochemical reactions for many enzymes and proteins

Patient No	Week	Se ($\mu\text{g/L}$) Mean \pm SD	Ca (mg/L) Mean \pm SD	Fe (mg/L) Mean \pm SD	K (mg/L) Mean \pm SD	Mg (mg/L) Mean \pm SD	Pb ($\mu\text{g/L}$) Mean \pm SD	Zn ($\mu\text{g/L}$) Mean \pm SD
1	1	6.387 \pm 0.415	2.604 \pm 0.023	4.737 \pm 0.233	5.342 \pm 0.365	0.780 \pm 0.045	0.458 \pm 0.015	0.124 \pm 0.015
	2	6.257 \pm 0.265	2.196 \pm 0.037	2.120 \pm 0.167	6.569 \pm 0.387	0.757 \pm 0.078	0.590 \pm 0.035	0.248 \pm 0.019
	3	6.260 \pm 0.562	2.485 \pm 0.048	4.384 \pm 0.197	4.723 \pm 0.560	0.921 \pm 0.065	0.695 \pm 0.037	0.168 \pm 0.024
	4	6.905 \pm 0.367	3.474 \pm 0.054	No observed	1.425 \pm 0.433	0.367 \pm 0.054	0.938 \pm 0.045	0.078 \pm 0.033
2	1	6.910 \pm 0.612	2.373 \pm 0.026	13.42 \pm 0.250	6.612 \pm 0.423	0.920 \pm 0.085	0.017 \pm 0.016	0.226 \pm 0.056
	2	6.636 \pm 0.455	2.502 \pm 0.140	6.315 \pm 0.167	4.971 \pm 0.388	0.737 \pm 0.034	0.013 \pm 0.011	0.131 \pm 0.060
	3	6.576 \pm 0.543	2.489 \pm 0.079	9.460 \pm 0.188	5.412 \pm 0.548	1.224 \pm 0.145	0.501 \pm 0.019	0.140 \pm 0.056
	4	6.43 \pm 0.397	2.328 \pm 0.063	8.042 \pm 0.245	5.485 \pm 0.275	1.432 \pm 0.187	0.621 \pm 0.032	0.151 \pm 0.047
3	1	7.067 \pm 0.451	3.632 \pm 0.075	3.597 \pm 0.195	3.427 \pm 0.197	0.545 \pm 0.054	0.033 \pm 0.013	0.200 \pm 0.054
	2	6.841 \pm 0.432	2.491 \pm 0.042	4.722 \pm 0.345	3.624 \pm 0.376	0.739 \pm 0.076	0.022 \pm 0.009	0.110 \pm 0.089
	3	6.093 \pm 0.765	2.761 \pm 0.049	2.874 \pm 0.155	3.829 \pm 0.675	0.638 \pm 0.098	0.023 \pm 0.006	0.070 \pm 0.088
	4	5.456 \pm 0.227	2.901 \pm 0.065	1.687 \pm 0.164	3.623 \pm 0.377	0.589 \pm 0.048	0.027 \pm 0.010	0.030 \pm 0.045
Ref. 1		3.650 \pm 0.17	13.48 \pm 0.38	3.61 \pm 0.15	15.39 \pm 0.36	14.88 \pm 0.10	1.74 \pm 0.19	4.44 \pm 0.15
Ref. 2		4.17 \pm 0.06	13.70 \pm 0.30	4.78 \pm 0.10	15.10 \pm 0.14	18.53 \pm 0.39	0.01 \pm 0.01	5.09 \pm 0.25

Table 1: Comparison of trace elements in serums taken from different patients with lung cancer.

Patient No	Week	Se ($\mu\text{g/L}$) Mean \pm SD	Ca (mg/L) Mean \pm SD	Fe (mg/L) Mean \pm SD	K (mg/L) Mean \pm SD	Mg (mg/L) Mean \pm SD	Pb ($\mu\text{g/L}$) Mean \pm SD	Zn ($\mu\text{g/L}$) Mean \pm SD
1	1	7.026 \pm 0.467	2.991 \pm 0.023	4.590 \pm 0.033	8.085 \pm 0.690	0.697 \pm 0.017	0.022 \pm 0.019	0.067 \pm 0.015
	2	6.977 \pm 0.643	3.206 \pm 0.056	3.507 \pm 0.025	7.214 \pm 0.587	0.5190.067	0.012 \pm 0.013	0.019 \pm 0.012
	3	5.714 \pm 0.389	4.046 \pm 0.089	3.056 \pm 0.047	6.914 \pm 0.450	0.487 \pm 0.099	0.026 \pm 0.013	0.025 \pm 0.008
	4	4.618 \pm 0.564	4.965 \pm 0.054	2.367 \pm 0.76	6.523 \pm 0.632	0.387 \pm 0.087	0.024 \pm 0.015	0.038 \pm 0.011
2	1	6.835 \pm 0.786	2.626 \pm 0.076	5.650 \pm 0.037	4.662 \pm 0.941	0.729 \pm 0.064	0.015 \pm 0.009	0.140 \pm 0.007
	2	6.201 \pm 0.654	2.777 \pm 0.123	4.867 \pm 0.024	5.148 \pm 0.754	0.987 \pm 0.078	0.012 \pm 0.007	0.032 \pm 0.005
	3	5.872 \pm 0.433	2.8920.079	3.690 \pm 0.065	6.130 \pm 0.367	1.176 \pm 0.086	0.010 \pm 0.008	0.013 \pm 0.015
	4	4.768 \pm 0.586	2.987 \pm 0.073	2.564 \pm 0.041	7.450 \pm 0.578	1.289 \pm 0.068	0.085 \pm 0.011	0.012 \pm 0.004
3	1	6.847 \pm 0.387	3.467 \pm 0.067	3.623 \pm 0.069	6.540 \pm 0.654	0.457 \pm 0.098	0.015 \pm 0.005	0.079 \pm 0.013
	2	4.849 \pm 0.761	4.394 \pm 0.084	1.269 \pm 0.058	6.858 \pm 0.438	0.420 \pm 0.054	0.026 \pm 0.013	0.081 \pm 0.017
	3	6.814 \pm 0.659	2.726 \pm 0.099	0.954 \pm 0.066	6.022 \pm 0.854	0.409 \pm 0.075	0.016 \pm 0.001	No detected
	4	5.643 \pm 0.588	3.824 \pm 0.058	0.780 \pm 0.053	5.908 \pm 0.347	0.380 \pm 0.063	0.012 \pm 0.006	No detected

Table 2: Comparison of trace elements in serums taken from different patients with throat cancer.

Patient No	Week	Se ($\mu\text{g/L}$) Mean \pm SD	Ca (mg/L) Mean \pm SD	Fe (mg/L) Mean \pm SD	K (mg/L) Mean \pm SD	Mg (mg/L) Mean \pm SD	Pb ($\mu\text{g/L}$) Mean \pm SD	Zn ($\mu\text{g/L}$) Mean \pm SD
1	1	7.436 \pm 0.667	4.474 \pm 0.067	5.659 \pm 0.036	62.92 \pm 0.65	0.686 \pm 0.056	0.018 \pm 0.012	0.247 \pm 0.012
	2	7.054 \pm 0.357	3.609 \pm 0.087	4.876 \pm 0.045	101.05 \pm 1.99	0.569 \pm 0.064	0.019 \pm 0.014	0.370 \pm 0.017
	3	6.681 \pm 0.653	3.633 \pm 0.067	4.056 \pm 0.054	78.95 \pm 3.87	0.435 \pm 0.98	0.043 \pm 0.024	0.182 \pm 0.011
	4	6.428 \pm 0.873	3.657 \pm 0.088	2.568 \pm 0.076	72.63 \pm 6.78	0.356 \pm 0.075	0.044 \pm 0.011	0.196 \pm 0.090
2	1	6.869 \pm 1.087	3.042 \pm 0.046	5.786 \pm 0.067	76.66 \pm 0.65	0.608 \pm 0.68	0.026 \pm 0.015	0.036 \pm 0.015
	2	7.390 \pm 0.786	3.259 \pm 0.064	4.467 \pm 0.043	78.13 \pm 4.89	0.55 \pm 0.84	0.021 \pm 0.009	0.059 \pm 0.012
	3	6.155 \pm 0.679	4.349 \pm 0.058	3.285 \pm 0.088	68.01 \pm 3.87	0.338 \pm 0.063	0.019 \pm 0.011	0.039 \pm 0.017
	4	5.453 \pm 0.975	5.231 \pm 0.078	2.154 \pm 0.051	66.94 \pm 7.45	0.276 \pm 0.045	0.016 \pm 0.007	0.029 \pm 0.012
3	1	6.9930.678	3.956 \pm 0.059	4.242 \pm 0.064	85.32 \pm 3.58	0.565 \pm 0.079	0.023 \pm 0.012	0.054 \pm 0.019
	2	6.744 \pm 0.543	3.123 \pm 0.047	2.369 \pm 0.055	62.39 \pm 5.66	0.511 \pm 0.054	0.021 \pm 0.009	0.033 \pm 0.013
	3	5.956 \pm 0.865	3.166 \pm 0.076	1.843 \pm 0.092	69.03 \pm 4.78	0.315 \pm 0.013	0.020 \pm 0.011	0.028 \pm 0.015
	4	5.354 \pm 0.673	3.043 \pm 0.054	1.567 \pm 0.043	72.012.89	0.310 \pm 0.068	0.019 \pm 0.006	0.025 \pm 0.013

Table 3: Comparison of trace elements in serums taken from different patients with stomach cancer.

used for controlling tumor growth Smoking may affect the amount of trace element levels in serums because of the variation in the amount of trace elements among cigarette brands. Variation in the levels of trace elements including Pb may depend on the inflammatory response attributed to brands of cigarettes. The number of cigarettes smoked and brands of cigarette increase the cigarette content in the serum of smokers, affecting the amounts of trace elements in serum and responding to medical treatment. In preventing cancer, one needs to choose nutrients which augment any of the cellular and organismic defense mechanisms reviewed. This is the foundation for the claim that many enzymes and proteins containing the trace elements used to control tumor growth and/or preventing cancer. In this study, the data which suggest that decreasing the amounts of Se, Ca, Fe, K, Mg, and Zn is associated with increased cancer are reviewed. Several hypotheses have been proposed to explain the effects of various chemotherapy based on its different biochemical activities, but not on the amount of trace elements in serums in pre-and-post chemotherapy periods. The variations in the amounts of elements for post-chemotherapy are because of either the cancer itself and/or previous treatments patients had taken before chemotherapy. Cancer leads to low trace element status, which supports the results presented in this study. In conclusion, the results of the present study confirm the decrease in most of the trace elements in cancer patients after chemotherapy. The decreases in the amounts show that chemotherapy destroys and/or damages body resistance and digestive system. The unwanted cases cause a decrease in biochemical reactions for many enzymes and proteins used to control tumor growth. The present study confirms that anticancer treatment is not appropriate for biological enzyme systems. However, further studies are still necessary to evaluate confirmation of lowering the amounts of trace elements by cancer and anticancer drug toxicity on digestive system, liver, and the other organs. The quantities

Patient No	Week	Ca/Mg	Ca/K	Se/Fe ($\times 10^{-3}$)	Se/Zn	Se/Pb
1	1	3,338	0.487	1.348	51.508	13,945
	2	2,928	0.334	2.951	25.229	10,587
	3	2,698	0.526	1.428	37.261	9,007
	4	9,466	2	3.815	44.538	7,361
2	1	2,579	0.359	0.515	30.575	40,600
	2	3,394	0.503	1.05	50.656	51,000
	3	2,033	0.46	0.695	46.97	13,127
	4	1,625	0.424	0.799	42.596	10,357
3	1	6,664	1	1.964	35.335	214,000
	2	3,938	0.687	1.448	62.191	309,000
	3	4,327	0.721	2.12	87.043	264,000
	4	4,925	0.8	3.234	181.186	202,000

Table 4: Variation ratio of elements with time for lung cancer.

Patient No	Week	Ca/Mg	Ca/K	Se/Fe ($\times 10^{-3}$)	Se/Zn
1	1	4.291	0.370	1.531	104
	2	11.904	0.444	1.989	367
	3	8.141	0.585	1.869	229
	4	12.829	0.761	1.951	122
2	1	3.602	0.563	1.209	49
	2	2.814	2.814	1.274	193
	3	2.459	0.471	1.591	451
	4	2.317	0.401	1.859	397
3	1	7.586	0.530	1.890	Uncalculable
	2	10.462	0.641	3.821	Uncalculable
	3	6.665	0.453	7.142	Uncalculable
	4	10.063	0.647	7.234	Uncalculable

Table 5: Variation ratio of elements with time for throat cancer.

of trace elements in serum decreases with their absorptions by body. The values given in the tables are the mean values of the data obtained from different patients with the same cancer types. Since the result obtained from different patients varies from each other due to fact that each patient's response to treatment was different. This is because each patient has a different degree and resistance to the disease, different body postures and periods of treatments. The ratios of the elements given in Tables 4 and 5 also decreased. However, this study clearly shows the fact that chemotherapy does not augment the rates of macro and trace elements. As the time passes the anti-cancer activities, synthesis of nucleic acids and proteins, and the structural stability diminish day by day. Consequently, the scientific approach in cancer therapy must be organized according to the stage, period of treatment and the physical state of the patients. Such changes in treatment of the cancer patients should be individually evaluated with continuing treatment according to their test results. Briefly, the characteristics of each patient are different from others. Considering the genetic impact on cancer, the question arises as to why other members of the family sharing the same environment do not have cancer. This is one of the major question to be answered. If genetic factors and lifestyle has a certain influence on the diseases, one would expect that all of the family members would suffer from the same diseases.

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