Short communication

Determination of Selenium as a Biomarker of Thyroid Cancer by HG-AFS Method

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Abstract

A simple and versatile procedure has been developed for the determination of selenium in biological samples for clinical purposes. The procedure consists of microwave sample digestion and the determination of selenium using atomic fluorescence spectrometry with a hydride generation system (HG-AFS). The method allows the determination of selenium in a range of 0.5–100.0 μg L^{-1} with a detection limit not higher than 0.2 μg L^{-1} and with good repeatability not exceeding 1%. It was applied for determination of selenium in women's plasma samples (n = 90) with a suspicion of a thyroid cancer and the control group of women (n = 87). Most of the obtained results were in the range 30.0–60.0 μg L^{-1} and either did not match the physiological level of selenium in human plasma or indicate moderate selenium deficiency. In the further examinations the thyroid tissues taken from 30 patients were analyzed. In most of the cases the selenium concentration was found to be lower (0.14–1.67 μg g^{-1} wet weight) than its physiological level in a healthy tissue. On this basis the hypothesis has been drawn that selenium can be considered as an additional marker of the thyroid cancer disease.

Keywords: Selenium; thyroid cancer; atomic fluorescence spectrometry

1. Introduction

Food is the main source of easily available selenium for living organisms. Concentration of selenium in plants depends on the amount of this element present in the soil and water, as well as on the level of environmental pollution.[1]

Epidemiologic investigations showed that the frequency of tumor occurrence were significantly higher in the regions typified by low selenium concentration in soil than the others.[2] Too low concentration of selenium in human tissues increases the risk of cancer but probably does not have any influence on the size and the localization of pathological changes.[3,4] The examination carried out with many patients proved that in some cases of cancer (e.g. lung, breast, or prostate cancer) high selenium intake reduces morbidity and mortality caused by this disease.[5–8] Similarly, it was found that 200 μg of selenium supplemented daily from yeast for four years or more led to significant reduction (more than 60%) in case of prostate, skin, and colorectal cancers.[9,10] Moreover, Sundaram et al. who studied the malignant neoplasm of the brain stated that selenium was able to inhibit the growth of the tumor cells causing their apoptosis. Authors suggested that selenium or non-toxic selenium compounds could be some of the most important prevention agents in the case of brain tumor.[11]

It was found that in the malignant neoplasm of breast or lung the selenium concentration was 1.7 times higher comparing to healthy adjacent tissues. Although the mechanism of selenium accumulation in tumor tissue is still not well recognized, it is known that this effect is connected with the process of induction of synthesis of GSH-Px (glutathione peroxidase enzyme), which contains selenium in its active center. This enzyme is one of the primary antioxidants hence it prevents various pathological changes in internal organs.[12]

On the other hand, the research carried out in Keshan region, China (where the selenium intake is lowest over the world) showed the inverse correlation between the selenium concentration in blood and the incidence of the cancer disease of lung, stomach, esophagus and liver.[13] In another research it has been stated that the concentration of selenium in body fluids of patients with tumors was lower in most cases, comparing to healthy persons.[1–5]

The diverse changes of selenium concentration mentioned above could be connected with specific properties of selenium. This element is considered to be the 'most toxic among essential elements' as the difference between its therapeutic and toxic level is very small.[14] This is the reason for which the appropriate assessment of the role of selenium in human organism requires an analytical tool. It should be capable of determining this element in tissues with especially good reliability in terms of accuracy and precision. As the concentration of selenium in tissues is very low[15–18] (see Table 1), the analytical technique applied has to be also characterized by high sensitivity and a low limit of detection. Nowadays a lot of analytical methods are used to determination of selenium and its speciation.[19–25] Furthermore, due to the complexity of the matrix of biological samples the calibration strategy should be carefully taken into account.[26]

Accuracy of selenium determination in biological samples mostly depends on the efficiency of its transfer from an organic matrix to solution. Currently, the most popular method of decomposition of organic matrix is microwave digestion[27] (see Table 2). However, the described procedures usually require the use of many reagents and to apply multistage digestion processes in order to eliminate interferences. As a consequence, they become expensive and laborious. In addition, they provide many sources of both random and systematic errors connected with possible contamination of reagents and/or a loss of an analyte at each individual procedural stage.

Table 1. The average content in tissues in the different organs for various populations.[15–18]

Organs	Physiological amount of selenium μg g^{-1}			
	Poland	**Germany**	**Italy**	**Bulgaria**
Liver	0.54	0.29	1.60	0.82
Spleen	0.34	0.23	0.90	0.78
Kidney	0.61	1.09	0.77	4.78
Lung	0.15	0.13	1.16	*
Brain	0.13	0.11	*	*
Heart	*	0.28	*	0.63
Pancreas	0.30	*	*	*
Testicles	*	0.30	*	*

* no data available

Table 2. Specification of digestion mixtures used for decomposition of various biological samples.[27]

No.	Biological samples	Required reagents	Analyte	Methods of analysis
1.	bovine liver*, hair, nails	HNO_3-$HClO_4$ **	As, Co, Cr, Cu, Ni, Pb, **Se**, Zn	AAS, NAA
2.	serum*, bovine liver	HNO_3-H_2SO_3, next H_2O_2	As, **Se**	HG-AAS
3.	pork liver	HNO_3-H_2O_2	Fe, Mn, **Se**	F-AAS, HG-AFS
4.	animals muscle*, food samples*	HNO_3 with add. 9 ppm Y (internal standard)	As, Ba, Fe, K, Mo, Ni, Pb, **Se**, Sr, V, Zn	PIXE
5.	bovine liver*, food samples*, sediment	HNO_3-HCl, next H_2O_2	B, Ba, Hg, Mn, Ni, Pb, Sb, **Se**	ICP-AES, ET-AAS
6.	blood*, urine*, bovine liver and muscle*	HNO_3-$HClO_4$- H_2SO_3	As, Cd, Co, Cu, Ni, **Se**,Pb	HG-AAS, DPASV
7.	oyster tissue*, human and animals muscles*	HNO_3, next H_2O_2	Al, As, Ba, Ca, Cd, Mg, Mn, Ni, Rb, **Se**, Sr i inne	ICP-MS
8.	pork kidney*, bovine liver*	HNO_3	Ag, Cd, Cu, Fe, Ga, Hg, Mn, Pb, Sb, **Se**	ZE-ET-AAS, ET-AAS, FANES
9.	crabs*	(1) HNO_3-$HClO_4$ (2) HNO_3-$HClO_4$- HF	As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, **Se**	ET-AAS, ICP-AES, F-AAS, ICP-MS, ASV

* samples of reference materials, ** digestion was made in the open system.

The purpose of this work is to present a simple, fast and inexpensive analytical procedure dedicated to the determination of selenium in biological samples. Reliability of the method has been verified by analyzing the reference materials of several biological materials. The method was applied to the determination of selenium in women's plasma with thyroid disorders. Based on the results obtained the attempt was made to estimate the relation between the occurrence of thyroid tumors and selenium concentration in this organ.

2. Experimental

2. 1. Reagents and Samples

Standard stock solutions containing 1000 mg L^{-1} Se was prepared from Titrisol standards (Merck, Germany). The working solutions were made with the use of concentrated HNO_3 (Merck, Germany), concentrated HCl (POCh, Poland) and 98% (m/v) $NaBH_4$ (Sigma-Aldrich Chemise, Germany), 0.5% (m/v) NaOH (Sigma-Aldrich Chemise, Germany). All reagents were of analytical reagent grade. Doubly deionised water (< 1.0 μS cm^{-1}) was use throughout.

The following reference materials were examined: human blood (LOT 404109, level 3, Norway), human serum (MI01181, level 1, Norway), bovine liver (SRM 1577b, Germany) pig kidney (BCR 186, Belgium), pork muscle (GBW 08552, China)

Plasma samples were obtained from the Blood Bank in Krakow. Samples were taken from persons of good health and not taking a selenium supplement. Plasma samples and extracts of pathogenic thyroid tissues (the study group) were obtained from patients with thyroid tumors, from The Third Department of General Surgery, Collegium Medicum in Krakow. The plasma and thyroid tissue samples were stored frozen in polypropylene bottles at –20 °C until analysis.

2. 2. Instrumentation

The microwave system MARS X (CEM, Matthews, USA) was used for sample digestion. An internal control system allowed programmable control of the pressure and temperature up to 5520 kPa (800 psi) and 220 °C, respectively. The specific parameters of the digestion process were given in Table 3. To remove gas from above digested sample with the nitrogen stream, the Mini-vap device was used (Sigma Aldrich, Germany).

A double-channel atomic fluorescence spectrometer AFS-230 (Beijing Haiguang Instrument Co., China), equipped with a 130-positional autosampler and a flow hydride-generation system with an intermittent flow method, was used for the measurement of analytical signals. The light sources used were cathode lamps (Se-HCL), specially dedicated for AFS measurements. Operating current (pulsed value) of these lamps was 100 mA. Argon was used as the shield and the carrier gas with a flow of 800 and 500 mL min^{-1} respectively. The atomization process occurred in the Ar–H_2 flame at the temperature of 200 °C. The signals were measured for selenium and processed in the peak area mode with the use of the IBM 586 computer.

Statistical calculations were carried out using the commercially available package Statistica v. 6.0. (StatSoft, Polska).

3. Results and Discussion

3. 1. Sample Preparation

Samples were digested according to the procedure developed in our laboratory.[28,29] Not more than 0.5 ml (body fluid) or 0.5 g (solid tissue) of sample was placed in a high-pressure teflon vessel, 7 mL of concentrated HNO_3 was added and then the vessel was closed and transferred into the microwave oven for digestion. The program of mineralization was described in details in Table 3. After digestion the vessel was cooled to the temperature of 25 °C. Then it was opened and the gas above the sample was removed (see below for details) by a stream of nitrogen gently flowing for several minutes. Then the sample was transferred into a 25 mL volumetric flask, and 12.5 mL of 6 mol L^{-1} hydrochloric acid (as the pre-reduction reagent) was added and the solution was diluted to the mark with water.

Before introducing the solution into the AFS instrument it was merged with a solution containing 2% (m v^{-1}) sodiumtetrahydroborate and 0.5% (m v^{-1}) sodium hydroxide in the flow hydride generation system. Hydrochloric acid in a concentration of 3 mol L^{-1} was used as a carrier solution.

Three portions of each sample were digested at the same time. Together with these, the blank (i.e. the solution containing all the required reagents except sample) was digested and then treated like a sample. Fluorescence for selenium was measured as the difference between the signals produced by the sample and by the blank.

Table 3. Parameters of the optimized digestion procedure.

Stage	Power %	Temperature °C	Pressure psi	Ramp time min.	Hold time min.
I	90	180			
II	90	200	350	4.00	4.00
III	95	220			

3. 2. Analytical Aspects

The serious problem dealing with the determination of selenium in the form of hydrides is a strong negative influence of nitrogen oxides on the analytical results.[30,31] The cause of this effect was reported to be based on the fact that nitrogen oxides prevent the reduction of analyte ions to a compound that is capable of forming hydrides with the highest efficiency. In order to avoid this effect it is recommended to use a way that was proved to be efficient in analyses of other biological materials:[28,29,32] to remove nitrogen oxides from the mineralized sample using the stream of an inert gas (argon, nitrogen) at pressure of 5 bar for a few minutes. The results shown in Table 4 revealed that selenium can be determined in various reference materials with good accuracy and precision when nitrogen is used for 10 minutes.

The analytical range was found to be linear up to100 μg L^{-1}. The calibration can be performed with use of inorganic standard solutions without their prior digestion or other special treatment before measurements. The limit of selenium detection in the biological samples examined was defined to be 0.2 μg L^{-1}.

Table 4. Results of the determination of selenium in the reference samples obtained in different conditions defined by the time of the removal of nitrous oxides by using the nitrogen stream.

Materials	Expelling time min.	Concentration of Se μg g^{-1}	
		Certified	Found
Blood	0	79 ± 1.5	64.5 ± 5.5 *
	5		71.5 ± 5.1
	10		81.5 ± 1.4
Serum	0	80 ± 2.5	69.5 ± 5.4
	5		71.5 ± 4.1
	10		79.5 ± 1.3
Pig kidney	0	10.3 ± 0.5	< DL
	5		9.86 ± 0.01
	10		9.94 ± 0.01
Pork muscle	0	0.49 ± 0.05	0.246 ± 0.006
	5		0.446 ± 0.003
	10		0.492 ± 0.002
Bovine liver	0	0.73 ± 0.06	0.532 ± 0.081
	5		0.701 ± 0.012
	10		0.721 ± 0.003

* confidence interval was calculated according to formula: $\Delta x = t(\alpha,f)s/\sqrt{n}$ where $\alpha = 0.05$, $f = 2$, $n = 3$ and s is the standard deviation

3. 3. Application: Selenium Status and Cancer Disease – Case Studies

As mentioned in the introduction, numerous clinical and epidemiological studies proved that the level of selenium in human body fluids and tissues affects the risk of the occurrence of some cancers, especially thyroid cancer, which is the most common endocrine cancer.[33–36] The aim of this part of the research presented here was to confirm that thesis by means of the analytical method developed.

First, the analytical procedure was used to determine selenium in the first group of women at risk of thyroid cancers. The 90 plasma samples were analyzed. All samples were collected from patients before eating. In the same time and using the same conditions 87 samples of plasma taken from the other group of healthy women (the control group) were analyzed. As the samples' volumes were small, each of them was analyzed only once. The results of these experiments are shown in the Fig. 1. In addition, the average physiological level of selenium determined on the basis of literature data is also shown in Figure 1.[37]

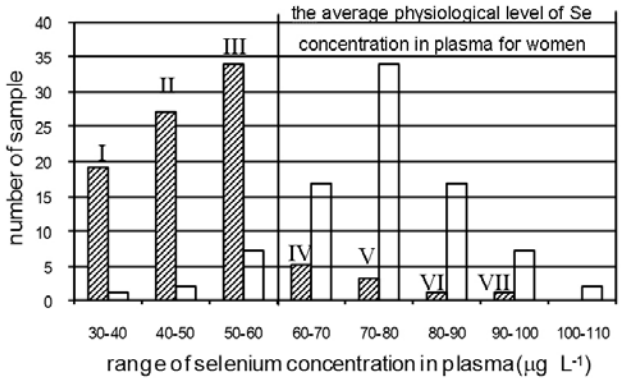

Figure 1. Selenium level in plasma samples collected from women with suspicion of cancer (hatched columns) and the control group of women (empty columns). The roman numbers present the groups of patients with different ranges of selenium level.

The results obtained for the whole study group were assigned to subgroups of patients (from I to VII). Each subgroup was characterized by a particular range of selenium concentration in plasma. The majority of the obtained results did not match the physiological level of selenium in human plasma. Only in 10 cases in 90 (group IV–VII) the patients had normal amount of selenium. In the remaining groups of patients the selenium status was detected to be very low (I–III, 89% of all patients). In addition, the selenium level determined in 24% of patients (group I–III) was so low as 30–40 μg L^{-1}.

Further tests were concerning the determination of selenium in thyroid gland tumors. According to available data the pathology of the thyroid may be associated with iodine and also selenium deficiency.[33,36] Therefore, many research institutes decided to determine selenium concentration in blood or plasma and, at the same time in thyroid tissues of patients with various thyroid diseases: nodular goiter, thyroid cancer and Grave's diseases. Similar research was carried out in the group of patients with hyperthyroidism, cancer and thyroid adenoma. In all cases the selenium level was lower than average and the lowest level was observed in thyroid tissues taken from patients with cancer.[37]

Thyroid tissue samples in present work were taken during surgical operations on 30 patients from Collegium Medicum UJ, Krakow. In most cases amounts of tissues were very small and were ranged from 0.07 to 0.5 grams.

The patients were diagnosed with: *struna nodosa*; *Graves' disease or toxic nodular goitre*; *follicular or macrofollicular adenoma*; *papillary carcinoma*; *lymphoma maligant*. The selenium concentration results ranged from 0.14 to 1.67 μg g^{-1} (wet weight). The results assigned into the subgroups are shown in Figure 2. They show that in most patients (90% of study group) the selenium concentration was found in thyroid tissues to be lower than the physiological level typical for healthy tissues (i.e, ca 1.24 μg g^{-1}). Moreover, in 50% of patients selenium was determined in very low concentration (less than 0.4 μg g^{-1}).

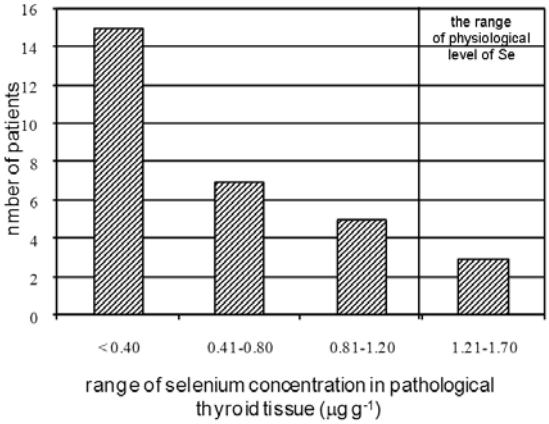

Figure 2. Number of patients and the range of selenium concentration.

Figure 3 illustrates the relation between selenium levels in thyroid tissues and the types of diseases the patients were diagnosed with. Kruskal-Wallis test was applied in order to check for any differences between various groups of patients and to find the possible relation between selenium level and diagnosed diseases. Differences with $p < 0.05$ were considered to be statistically significant. The analysis did not reveal statistically significant differences between studied groups ($p = 0.374$).

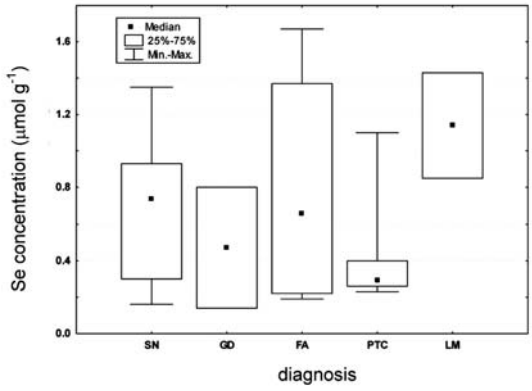

Figure 3. The relation between selenium levels in thyroid tissues and diagnosis (SN – struna nodosa; GD – Graves' disease or toxic nodular goitre; FA – follicular or macrofollicular adenoma; PTC – papillary carcinoma; LM – lymphpma maligant).

4. Conclusions

Interesting papers showing the influence of selenium on human health encourage many authors to intensify their study on the biological function of selenium and its influence on the human body. Obtaining reliable information on this subject, is in a large measure depending on the selection and skilful application of proper analytical methods.

In the presented study a simple and low cost method was shown which gives a great possibility to determine selenium with good accuracy and precision at the low selenium levels (at physiological levels and lower) in various biological materials. The reliability of this method was successfully confirmed many times by analysis of a great number of various reference materials.

It was proved that the presented method can be used to determine selenium in the human tissues taken from people suffering from cancer. The obtained results of plasma samples and thyroid tissues show the association between selenium level and a risk of cancer. Moreover, the results proved the hypothesis that a low selenium level is characteristic for thyroid cancer and it can be a „marker" of this kind of disease. The research also showed that in the group of women with a risk of cancer the revealed plasma selenium levels were significantly lower than physiological level. However, it was not proved that there is any association between selenium concentrations in pathogenically changed tissues taken from an examined group with types of thyroid disease. It is possible that the correlation could be proved on the basis of analytical examinations with participation of much greater number of patients.

5. Acknowledgement

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Povzetek

Razvili smo enostaven postopek za določanje selena v bioloških vzorcih. Vzorec razkrojimo s pomočjo mikrovalov ter nadaljujemo z določitvijo selena s pomočjo atomske fluorescenčne spektrometrije s hidridno tehniko (HG-AFS). Metoda je uporabna v območju 0,5–100 g L^{-1} z mejo določitve 0,2 g L^{-1} ter ponovljivostjo 1 %. Uporabili smo jo za določanje selena v krvni plazmi žensk (n = 90), pri katerih smo sumili prisotnost tiroidnega raka, ter pri kontrolni skupini (n = 87). Večina rezultatov je bila v območju 30–60 g L^{-1}, kar ne odgovarja fiziološkim koncentracijam selena v človeški plazmi ali kaže celo na pomanjkanje. V nadaljnjih raziskavah smo preverili še tiroidna tkiva 30 pacientk in ugotovili znižane koncentracije (0,14–1,67 g g^{-1} mokre mase). Na osnovi tega smo zaključili, da lahko selen uporabimo kot dodatni marker za prisotnost tiroidnega raka.