

URANIUM CONCENTRATIONS IN TISSUES AND OTHER CLINICAL SAMPLES OF THE SERBIAN POPULATION

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The prevalence of numerous malignant diseases is on the rise, while the mechanism of metal-induced oncogenesis has not been elucidated so far. The aim of this study was to determine the amount of uranium (U) in blood samples of the Serbian population ($n = 305$) and to perform a comparative analysis with the amounts of U in the blood of patients with thyroid carcinoma (TC, $n = 103$) and malignant brain tumors (MBTs, $n = 157$). This study also aimed to extend data on the tissue sample analysis. Uranium was quantified by inductively coupled quadrupole plasma mass spectrometry (ICP-Q-MS). The content of U was approximately 15 times higher in the Serbian population compared to other population groups worldwide that did not suffer from the war, while its amount showed similarities with the countries that directly suffered from the war. Furthermore, the U content was up to twice as high in the blood samples of TC patients compared to the control, while the U content in the TC tissue samples was approximately 10 times higher than in healthy thyroid tissues and showed a tendency to be higher in follicular variant of papillary thyroid carcinoma. However, the highest alterations in U content were obtained in samples of MBT patients, both in liquid clinical samples (serum, lysate, and cerebrospinal fluid) and in tissue samples. The results of this study could highlight the unresolved etiology of TC and MBT. Moreover, the reported results indicated the importance of regular monitoring of U in the blood of the Serbian population.

Keywords: Uranium, Serbian population, Thyroid carcinomas, Malignant brain tumors, Etiology.

Introduction

Uranium (U) is slightly radioactive and it can be found in the environment in bound form with silicates, carbonates, phosphates, etc. It occurs most often in the hexavalent state, which is characterized by uranyl ion [1–3].

Uranium occurs in nature in the form of three isotopes: ^{238}U (99.275%), ^{235}U (0.720%), and ^{234}U (0.0055%). The most radioactive is ^{235}U , which has a half-life of 7.038×10^8 years, followed by ^{234}U with a half-life of 2.455×10^5 years and ^{238}U with a half-life of 4.468×10^9 years. Many artificial isotopes of U were obtained so far of which the most important one is ^{233}U [4–7].

The isotope ^{235}U is most significant for nuclear energy. Considering that ^{238}U is the most abundant in nature, enrichment with a poorer isotope (^{235}U) needs to be carried out. The result of the enrichment process is the achievement of the desired percentage of the isotope ^{235}U (2–3%) from the original 0.72%, which is sufficient to achieve the quality of nuclear fuel. However, enrichment of U can achieve as much as 99% of the isotope ^{235}U with several thousand diffusion cycles. A by-product of the U enrichment process is depleted uranium (DU). Due to its high density and low cost, DU is used as an efficient material for the production of military weapons [7, 8–10].

In the last few decades, Europe has suffered several radioactive disasters, while Serbia (Federal Republic of Yugoslavia) directly suffered a war in 1999. Weapons with DU, which were used during airstrikes, are the main source of radioactive dust that was released in Serbia and the surrounding countries. According to the literature, more than 5 tons of DU was dumped on Serbia, while more than 10 tons were dumped on AP Kosovo and Metohija. Removal of DU at several locations in Serbia was carried

out in the period from 2002 to 2007. During the clearing of the terrain, corroded projectiles were found both on the ground surface and at depths greater than 1 m [11, 12].

Uranium is insoluble in water. However, when exposed to air or water, oxidation occurs and a large number of U oxides are formed. The oxidation rate is increased after direct contact DU penetrator with a solid surface (e.g. a military tank). The formed micrometer U-dust easily disperses in the environment and thus enters the human food chain [12, 14].

Uranium is introduced into the body by inhalation and/or ingestion. Uranyl ions in body fluids have a high affinity for complex formation, primarily with bicarbonates, which increases the solubility of U in blood plasma. Complex uranyl ions are excreted via urine in a relatively short period of time. However, a stochastic effect may occur during this period. The first organs to respond to U are highly radiosensitive tissues, such as bone marrow and lymph tissues, due to the short cell life and lack of an active system for repairing DNA molecules. The second axiom is the latent period, which implies the time from the action of the carcinogenic agent to the clinical manifestation of the malignant disease. For leukemia and lymphoma, the latent period is 5–10 years, while for solid tumors that period is 10–20 years. DU is slightly radioactive and its toxicity to human health is primarily chemical. Although bones and kidneys are the primary organs for U storage and the manifestation of the effects of its toxicity, it has been shown that this metal can easily cross the blood-brain barrier and accumulate in the brain tissue [10, 13, 16].

The aim of this study was to determine the U amount in the blood of a healthy Serbian population and to make a comparative analysis with the U amount in the blood of patients with TC and MBT. In addition to the short-term information that body fluids can provide, this study also aimed to extend data on tissue samples of the same patients to monitor long-term exposure.

Experimental

Sample collection

Venous blood was collected from blood donors ($n = 305$; f/m ratio = 163/142, age: 43 ± 8 years) and patients diagnosed with TC ($n = 103$; f/m ratio = 80/23, age: 44 ± 3 years) and MBT ($n = 157$; ratio f/m = 63/94, age: 37 ± 13 years). The blood of MBT patients was separated into serum and lysate after centrifugation ($3000 \times \text{rpm}$). Tissue samples were collected from the same patients during surgery. Malignant thyroid tissue (MTT) and healthy thyroid tissue (HTT) were collected during thyroidectomy. HTT was collected from the greatest possible distance from the primary tumor, which was well demarcated. The standard technique of surgical excision of the tumor involved the formation of the peritumoral margin of normal cerebral tissue. This tissue was collected and considered as healthy brain tissue (HBT) after the exclusion of a malignant tumor or other pathological entity. Pathohistological (PH) analysis of HTT and HBT samples did not reveal the presence of other pathological entity. From patients with MBT and hydrocephalus patients ($n = 117$; ratio f/m = 61/56, age: 34 ± 11 years), who formed an additional control group, cerebrospinal fluid (CSF) was collected by ventricular puncture during surgical implantation of the shunt. The highest number of TC patients had been diagnosed with a papillary thyroid carcinoma ($n = 66$), followed by follicular carcinoma ($n = 23$) and medullary thyroid carcinoma ($n = 14$). The greatest number of MBT patients had been diagnosed with grade III glioblastoma ($n = 111$), and the other malignant tumors, including grade III medulloblastoma ($n = 26$) and grade III astrocytoma ($n = 20$). All samples were stored at -80°C until analysis.

Patients with other malignant or chronic diseases, smokers and workers occupationally exposed to metals were excluded from the study. Approval for this study was obtained from the Ethics Committee of the Clinical Center of Serbia in Belgrade. All volunteers and patients voluntarily agreed to participate in this study and written consent was obtained from each person.

Chemicals, samples preparation and instrumentation

All samples were prepared as previously described [17]. Briefly, samples were weighed and digested in a microwave oven in a mixture of high-quality nitric acid (65%) and hydrogen peroxide (30%) (4:1, v/v) at 180°C . Decomposed samples were diluted into 25 mL-volumetric flasks with Milli Q water. ^{238}U isotope was determined by inductively coupled plasma quadrupole mass spectrometry (ICP-Q-MS) in standard mode. Very good linearity of the calibration curve was obtained in the range from 1 to $50 \mu\text{g/L}$ ($r > 0.99$). ICP-Q-MS accuracy was controlled by standard reference materials (SRM) of blood

(SERO210105-Level-1) supplied by Seronorm (Sero AS, Norway). The obtained recovery ranged from 92.0 to 106.2%.

Statistical analysis

All data were analyzed using SPSS statistical software (IBM SPSS Statistics 20). The distribution of data was checked with the Kolmogorov-Smirnov test. Since the results were not equally distributed, statistical differences between the groups were examined with the Mann–Whitney U test and presented graphically using box plots. In the tests used, the null hypothesis was rejected at the significance level $P < 0.05$. The reference interval for U was expressed in percentiles (P) in the range of 5 to 95% and it was calculated as lower limit (LL) and upper limit (UL) of the 95% confidence interval.

Results and discussion

The parameters of descriptive statistics for U amount (mean, st. dev., min., max., median) in the examined blood samples of a healthy Serbian population (total content and content based on differences in sex and age) are given in Table 1, together with the geometric mean (GM), selected Ps, LL, and UL. A comparative analysis of U amounts in the blood samples of a healthy Serbian population with amounts of U in blood samples of TC patients is shown in Fig. 1. The same figure also shows a comparative analysis of U amounts in HTT and MTT samples. The obtained results indicate that the amount of U in the blood of control samples and HTTs was similar, while the U amount in the blood of TC patients was twice as high on average. Furthermore, up to 10 times higher amounts in MTT were found compared to control ones ($P < 0.05$).

Table 1
Uranium amounts in the blood samples of a healthy Serbian population (ng/g)

Parameters		mean	st. dev.	min	max	median	GM	P5	P50	P95	LL	UL
Σ		0.06	0.04	0.03	0.20	0.05	0.06	0.03	0.05	0.13	0.05	0.07
Sex	Female	0.07	0.04	0.03	0.20	0.06	0.06	0.03	0.06	0.13	0.05	0.08
	Male	0.05	0.03	0.02	0.11	0.04	0.04	0.02	0.04	0.10	0.03	0.07
Age	< 45 years	0.07	0.05	0.03	0.20	0.05	0.06	0.03	0.05	0.14	0.05	0.09
	> 45 years	0.06	0.02	0.03	0.11	0.05	0.05	0.02	0.05	0.09	0.05	0.07

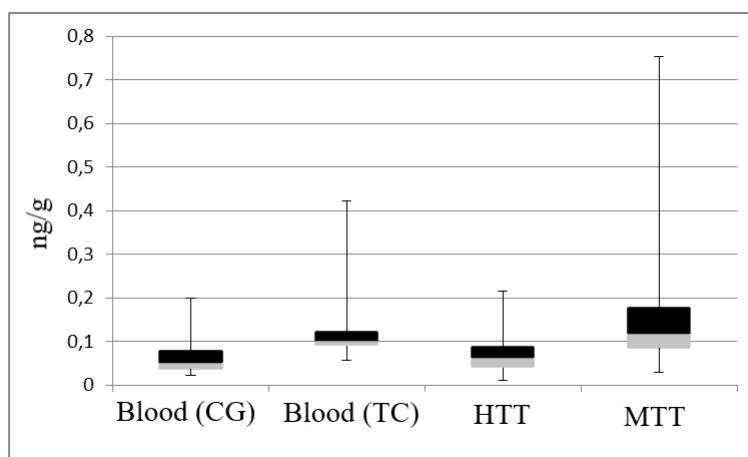


Fig. 1. Comparative analysis of uranium content in the blood and tissues of the control group (CG) and patients with thyroid carcinoma (TC); HTT – healthy thyroid tissue, MTT – malignant thyroid tissue

A comparative analysis of U amounts in serum, lysate, CSF, and tissue samples of MBT patients compared to control samples is shown in Fig. 2. A statistically significant difference in U amount was obtained between all study groups ($P < 0.05$). The highest alterations as well as the highest U amounts were found in serum samples and MBTs compared to the control ones. The results of this study also indicated that U can be detected in CSF samples at levels that are similar to its amounts in serum and lysate of control samples. Malignant CSF samples had about 2.5 times higher U amounts compared to

control samples of hydrocephalus patients. The most interesting information was obtained for the MBT samples since the values were up to 50 times higher compared to HBTs (Fig. 2).

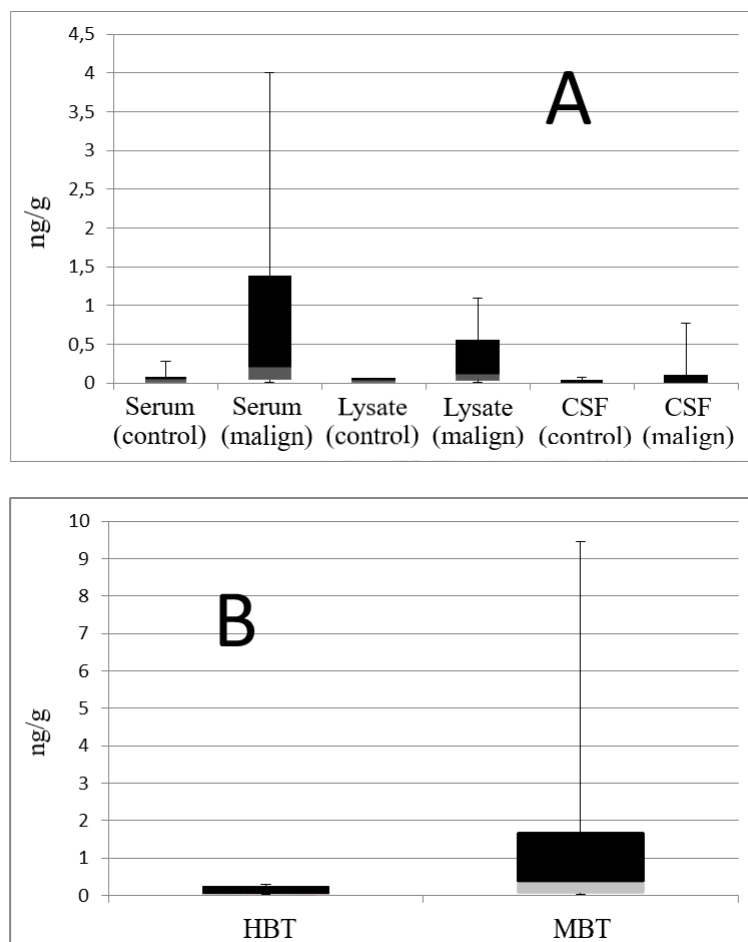


Fig. 2. Comparative analysis of uranium content in A) serum, lysate and cerebrospinal fluid (CSF) of patients with malignant brain tumors versus control; B) comparative analysis of U content between samples of healthy brain tissue (HBT) and malignant brain tissue (MBT)

In the last three decades, there has been a worldwide trend of establishing reference intervals for metals of clinical interest. Stojsavljević et al. [17] published the first reference interval for U in the blood samples of a healthy Serbian population. The samples were collected from 305 blood donors. The values obtained for U concentrations in the blood did not differ significantly according to sex and age of the subjects ($P > 0.05$). However, a comparative analysis with other population groups worldwide showed a significantly higher U amount in the Serbian population (0.06 ± 0.04 ng/g) compared to the countries that did not suffer from a war where weapons with DU were used. For example, Gouille et al. [18] reported a median U content of 0.004 ng/g for the French population, while Heitland and Koster reported a GM value of 0.003 ng/g for the German population [19], which was approximately 15 times lower than in the Serbian population.

Ferlay et al. [20] pointed out that TCs rank 24th in Serbia in incidence. Slijepcevic et al. [21] noted that the incidence of TC in Serbia for the period 1999–2008 was 3/100,000 for women and 1/100,000 for men. The highest prevalence was observed in women between 20 and 29 years and in men between 30 and 39 years. The authors also pointed out that an increase in new cases of TC could be expected in Serbia in the upcoming period.

The thyroid tissue is very sensitive to ionizing radiation, as evidenced by the consequences of the Chernobyl nuclear reactor accident in 1986, when approximately 1.7×10^{18} Bq ^{131}I was released into the atmosphere. One year after the Chernobyl catastrophe, a significant increase in the number of TC patients and other malignant diseases (leukemia, lymphoma, breast cancer, etc.) was registered in all age

categories, especially within a hundred kilometers of the power plant [5, 7]. The results of this study indicate that the amount of U in the blood and tissue samples of TC patients was significantly increased compared to the control group. An interesting data is the influence of U on the discrimination of the follicular PH variant of papillary TC from the solid variant (0.16 vs. 0.09 ng/g $P = 0.04$). The increased U amount in thyroid tissues with retrosternal growth was 0.24 ng/g versus 0.10 ng/g in thyroid tissues without retrosternal growth ($P = 0.01$), which can be explained by a longer period of accumulation of radionuclides in retrosternal thyroid goiter. Considering that uranium has been associated with different PH types of papillary thyroid carcinoma, it can be pointed out that amounts of U play a key role in activating different pathways of thyroid carcinogenesis.

Malignant brain tumors are among the most aggressive tumors and make up about 1.5% of all human cancers. The reported mortality from primary tumors of the central nervous system is 60% in the first five years. MBTs are most often diagnosed in men, especially in the elderly population. The most common type of brain tumors is gliomas and they occupy about 80% of all primary neoplasms [10, 14].

There are a large number of reported cases in the literature of an increased incidence of brain tumors in occupationally exposed people to U. Briner et al. [22] noted chromosome aberration and malignant alteration in workers exposed to DU, while two cohorts indicated a high risk of brain tumor incidence in people exposed to nuclear radiation ($n = 10,000$). Civit et al. [23] found that increased DU activity can cause DNA point mutations and carcinogenesis.

There are insufficient data in the literature on the concentration of U in blood samples, while its content in tissues and CSF is unknown. In the neighboring country, Croatia, Sarap et al. [24] reported serum U content in the range of 0.10-0.72 ng/g. Todorov et al. [25] analyzed the U content in the blood samples of veterans who were exposed to DU after the First Gulf War and reported the U content in the range of 0.14-0.80 ng/g. Civit et al. [23] collected blood samples from healthy people and patients with malignant diseases at several locations that were centers of intensive military activities during the First and Second Gulf Wars. The authors reported significantly increased values of U in the blood samples of cancer patients (2.62 ± 0.11 ng/g) compared to the control group (1.54 ± 0.1 ng/g). The values obtained in this study for serum (1.52 ± 0.68 ng/g) and lysate (1.27 ± 0.55 ng/g) are in good correlation with the reported values.

The increased U amount in body fluids, especially in MBTs, even in healthy ones, should be further examined. The most interesting and at the same time the most worrying information was obtained for the MBT samples, since the values were up to 50 times higher compared to HBTs. Considering that the ICP-Q-MS device used in this study only offers the possibility of determining the total amount of U in the analyzed samples, this study cannot provide data on whether DU was detected in tissue and body fluid samples. The main reason is the inability of the quadrupole to separate the ^{238}U isotope from the ^{235}U . This limitation can be easily solved by HR-ICP-MS.

Conclusion

It was found that the U amount in the blood of a healthy Serbian population was at least 15 times higher than in other countries worldwide that did not suffer a state of war with weapons from DU, and in correlation with countries that directly suffered a state of war with weapons from DU. The U amount was up to twice as high in the blood of TC patients compared to the blood of the healthy Serbian population. The amount of U in tissues with TC was about 10 times higher than in HTTs and showed a tendency to be higher in follicular variant of papillary thyroid carcinoma. The higher U amount was especially recognized for thyroid tissues with retrosternal growth. However, the most important changes in the amounts of U were observed in the samples of MBT patients, both in liquid clinical samples and in tissues. The results of this study could highlight the unexplained etiology of TC and MBT. Furthermore, reported results indicated the importance of regular monitoring of U in the blood of the Serbian population as well as in other population groups around the world.

Declaration of interest statement

The authors declare that they have no conflict of interest.

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КОНЦЕНТРАЦИЯ УРАНА В ТКАНЯХ И ДРУГИХ КЛИНИЧЕСКИХ ОБРАЗЦАХ НАСЕЛЕНИЯ СЕРБИИ

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Растет распространенность многочисленных злокачественных заболеваний, а вместе с тем механизм металл-индуцированного онкогенеза до сих пор не выяснен. Целью данного исследования было определение количества урана (U) в образцах крови сербского населения (численность выборки n = 305) и проведение сравнительного анализа количества U в крови пациентов с карциномой щитовидной железы (КЩЖ, n = 103) и злокачественными опухолями головного мозга (ЗОГМ, n = 157). Это исследование также было направлено на расширение данных об анализе образцов ткани. Количество урана определяли с помощью квадрупольной масс-спектрометрии с индуктивно связанной плазмой (ICP-Q-MS). Содержание U было примерно в 15 раз выше в сербском населении по сравнению с другими группами населения во всем мире, которые не пострадали от войны, в то время как его количество было сходным со странами, которые непосредственно пострадали от войны. Кроме того, содержание U было почти в два раза выше в образцах крови пациентов с КЩЖ по сравнению с контролем, в то время как содержание U в образцах тканей КЩЖ было примерно в 10 раз выше, чем в здоровых тканях щитовидной железы, и, как правило, было выше в образцах с фолликулярным вариантом папиллярной карциномы щитовидной

железы. Однако наибольшие изменения в содержании U были получены в образцах пациентов с ЗОГМ, как в жидких клинических образцах (сыворотка, лизат и спинномозговая жидкость), так и в образцах тканей. Результаты этого исследования могут выявить нерешенную этиологию КЩЖ и ЗОГМ. Более того, представленные результаты указывают на важность регулярного мониторинга U в крови сербского населения.

Ключевые слова: уран, сербское население, карцинома щитовидной железы, злокачественная опухоль головного мозга, этиология.

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