# BIOMONITORING OF NICKEL IN POPULATION OF ENDEMIC NEPHROPATHY SETTLEMENTS. A PRELIMINARY STUDY

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Endemic nephropathy (EN) is a disease of unkown etiology, and its occurrence is determined only at the Balkan peninsula. This study started form the assumption that nickel, being a highly toxic and cancerogenous substance, could be a risk factor or precipitating factor in generation of EN and urothelial tumors as well. The aim of the study was to prove the extent to which nickel, as a cancerogenous substance, is present in the urine and kidneys of inhabitants in EN settlements. As a material, 93 samples of urine and 32 samples of autopsy material were used. The urine samples were taken by the random sampling method from inhabitants of endemic and hypoendemic settlements in the valley of South Morava river, as well as from urban population of the municipality of Nis, as the control settlement. The samples of the kidney tissue represented autopsy material taken from cadavers who used to live, almost the whole life, in some of the endemic of hypoendemic settlements (target group), or in the urban zone of municipality of Nis (control group). The nickel presence was determined using method of atomic absorption spectrofotometry. The nickel concentration in the urine is higher in the endemic/hypoendemic settlements population than that in the control settlement group. The difference is not though, statistically significant. The nickel content in the kidney tissue of inhabitants from endemic or hypoendemic settlements is higher then in relevant kidney samples of inhabitants from control settlements, but the difference is not statistically significant either. Further investigations of the nickel content in biological materials are needed, along with the epidemiological study of incidence and prevalence of EN and malignant changes of the urotrackt in the endemic settlement population. *Acta Medica Medianae 2003; 42(3):15-18.*

*Key words: nickel, urine, kidney, endemic nephropathy*

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## Introduction

Endemic nephropathy (EN) is tubulointersticial disease of kidney unknown etiology and its occurrence is registered on th Balkan peninsula, in Bulgaria, Romania and republics of former Yugoslavia: Serbia, Bosnia and Croatia only. It is chronical and progressive kidney disease with onset in the third decade of the life and gradually leads to the terminal kidney failure. The disease is in connection with rural way of life and does not occur in the autochthonous urban population. The fact that newcomers of endemic regions may get the disease if they stay there long enough as well as the early leaving of the region that may prevent the onset of the disease, suggests importance of the environment in the etiology of EN. Some authors emphasize the greater incidence of the disease in female, while the others consider the sex not to be very important in the incidence and prevalence of the disease. The greater incidence of the urothelial tumors in endemic than in nonendemic neighbor regions is proved (1).

Many investigations were performed in the last decades in order to establish etiology of EN. Many factors were incriminated as genetic factors, microorganisms and their products (bacteria, viruses, micotoxins, plant products), non-living environmental factors (lead, cadmium, uranium, silicium), nutritional deficiency (deficiency in selenium) and many others. None of the mentioned hypothesis about dominant causes of this endemic disease was accepted by the scientific community so far (1, 2).

In this study we started from supposition that nickel, being highly toxic and cancerogenous substance, could be the risk and precipitating factor in generation of EN and urothelial tumors (1, 3,4, 5), and that urinary elimination is the most important way of elimination of nickel from organism in humans and most of the animal species (6).

**Aim** of **the study**

Aim of the study was to discover the extent to which the nickel, as a cancerogenous substance, is present in the urine and kidneys in population of EN settlements.

**Material and methods**

Laboratory material was represented with 93 samples of urine and 32 samples of autopsy material taken from the Institute for forensic medicine in Nis. The urine samples were taken randomly from population of endemic and hypoendemic settlements in the South Morava river valley, and from urban population of Nis municipality, as a control settlement. Only samples of adult persons who had spent almost the whole life in the settlement where the samples were taken, has been taken into consideration. Also, they were not professionally exposed to the nickel and didn't have signs of urotract disorders. Male examinees were 20-62 years of age (41 examinee, 16 from the endemic/hypoendemic regions, and 25 from the control settlements). Female examinees were 18-64 years of age (52 of them, 20 from endemic/hypoendemic regions and 32 examinees from control settlement).

Kidney tissue samples were autopsy material, taken from the corpses who used to live in some of endemic/hypoendemic villages for almost the whole life (target group), or in the urban zone of Nis municipality (control group). Just corpses of the persons died of natural or accidental death were taken into consideration. They also weren't professionally exposed to nickel or had data about urotract disorders. The autopsy samples were taken from 15 male corpses (56-71 years of age, 7 from endemic/hypoendemic settlements, and 8 from control settlements), and 17 female corpses (58-74 years of age, 7 of them from endemic/hypoendemic and 10 from control settlements).

The samples of urine taken in polyethylene cups previously rinsed with dejonised water and dried. After sampling biologic material was immediately proceeded to the laboratory of the Institute for Health Protection, Nis, where it was frozen and stored at -18°C. The presence of the nickel was determined using atomic absorption spectrofotometry method, graphite civet technique, on the Percin Elmer 100 spectrophotometer.

**Results and discussion**

Results of our study show presence and measurable concentrations of nickel in more than 75% of urine samples of men from endemic/hypoendemic settlements, and in more than 50% of urine samples of men from control settlements (table 1). The average value (1.62+58 u.gNi/1) of nickel concentration in urine of male from endemic/hypoendemic settlements was higher than that of male from the control settlements (1.37+1.68 ngNi/1). However, the difference between the two groups was not statistically important (p>0.05).

Nickel concentrations in male and female urine didn't differ much (table 2). The presence of nickel was registered in more than 50% of female both in the target and in the control group (Median was 1.00 p.gNi/1). The highest concentration was found in female from endemic settlements (8.2 p.gNi/1). The difference between the two groups was not statistically significant either (p>0.05).

Although very low in the biosphere, nickel can enter the organism by air, water, food, and by things for general use (7). After being absorbed, nickel reaches all the organs via blood, (the highest concentrations are found in detoxrcation organs such as kidney and liver).

There is little investigation in the world about biomonitoring of nickel in general population (8,9,10). However, there are many papers about occupationally exposed inhabitants, which indicates nefrotic effect chronical exposure to the nickel: diminished clearance, proteinuria, and decrease of the specific weight of urine. Experimental investigation also indicates nefrotoxic effects of the nickel (7).

*Table J.* The nickle content in urine of men from endemic/hypoendemic and control settlements

| Place of living | Numb of samples | Min | C25 | M | C75 | Max | X+SD | p* |
|---|---|---|---|---|---|---|---|---|
| | | ugNi/1 | | | | | | |
| Endemic/hypoendemic village | 16 | 0.00 | 0.25 | 1.20 | 2.20 | 4.60 | 1.62+1.58 | NS |
| Control settlement | 25 | 0.00 | 0.00 | 1.00 | 1.85 | 5.1 | 1.37+1.68 | |

* Mann-whitney Rank Sum Test

*Table 2.* The nickle content in urine of women from endemic/hypoendemic and control settlements

| Place of living | Numb of samples | Min | C25 | M | C75 | Max | X±SD | p* |
|---|---|---|---|---|---|---|---|---|
| | | UgNi/1 | | | | | | |
| Endemic/hypoendemic village | 20 | 0.00 | 0.00 | 1.00 | 2.50 | 8.20 | 1.91+2.38 | NS |
| Control settlement | 32 | 0.00 | 0.00 | 1.00 | 1.80 | 5.40 | 1.56+1.62 | |

* Mann-whitney Rank Sum Test

*Table 3.* The nickle content in kidneyurine of men from endemic/hypoendemic and control settlements

| Place of living | Min | CIO | M | C90 | Max | X±SD | p* |
|---|---|---|---|---|---|---|---|
| | ugNi/kg | | | | | | |
| Endemic/hypoendemic village | 0.086 | 0.094 | 1.145 | 0.158 | 0.160 | 1.154±0.008 | NS |
| Control settlement | 0.081 | 0.086 | 1.110 | 0.130 | 0.140 | 0.120±0.029 | |

* Mann-whitney Rank Sum Test

*Table 4.* The nickle content in kindeys of women from endemic/hypoendemic and control settlements

| Place of living | Min | C10 | M | C90 | Max | X±SD | p* |
|---|---|---|---|---|---|---|---|
| | ugNi/kg | | | | | | |
| Endemic/hypoendemic village | 0.122 | 0.140 | 1.148 | 0.167 | 0.197 | 1.163±0.025 | NS |
| Control settlement | 0.115 | 0.125 | 1.136 | 0.159 | 0.184 | 0.15810.034 | |

Mann-whitney Rank Sum Test

Results of our study show presence of measurable concentrations of nickel in all the examined autopsy kidney samples. The value of median (0.145 mgNi/kg), maximal value (0.160 mgNi/kg) as well as average value (0.154±0.008 mgNi/kg) of nickel concentration in the kidney tissue in male from endemic/hypoendemic settlement was higher comparing to the relevant values of male from the control settlements (M - 0.11 mgNi/kg, Max - 0.14 mgNi/kg, X±SD - 0.102+0.029 mgNi/kg). However, this difference was not statistically significant (table 3). In female, living in endemic/hypoendemic, higher bioconcentrations of nickel in kidney tissue were registered (M - 0.148 mgNi/kg, Max - 0,197 mgNi/kg, X±SD - 0.16310.025 mgNi/kg) compared to the biocentration of nickle in autopsy material of control group (M - 0.136 mgNi/kg, Max - 0.184 mgNi/kg X±SD - 0.158+0,034 mgNi/kg) but this difference was not important either (table 4).

There are also rare published papers about biomonitoring of nickle in EN patients. A group of Bulgarian scientists (4, 5) investigated concentrations of trace elements (Al, Sb, Ni, Cr) in autopsy material (kidney tissue and hair) in patients died of EN and those died with some other diagnosis who didn't live in endemic settlements. Higher concentrations of these elements in kidney tissue of EN patients compared with controls were established. Recorded concentrations of nickel in kidney tissue in EN patients were almost the same we found in our study (0.07-0.22 mgNi/kg).

The cancerogenous effect of nickel on urotract structures was proved in some papers dealing with occupational exposure to low concentrations of nickle during longer period of time. The cancerogenous effect of nickle was proved also on the molecular level (11). These investigations also proved protective role of antioxidants (ascorbic acid, selenium, magnesium and copper) during the action nickle on te structures of the cells (7).

Further investigations on eko and biomonitoring of the nickle in general population living in endemic settlements should certainly be performed on a larger number of samples in order to get more information about nickle as possible cause or precipitating factor in generation of EN and utothelial tumors. The results should be compared with kidney function of examinee and family distribution of mentioned diseases. Besides, the inhabitants of nonendemic rural settlements should be included, as a new, specific control group, in further investigations.

## Conclusion

**On** the basis of the results of investigations the following conclusions were made:

1. The nickle concentration in urine of endemic inhabitants is higher than that inhabitants from control settlements, although the difference is not significant.
2. Nickle content in kidney tissue of endemic inhabitants is higher than its concentration in relevant autopsy material of inhabitants from control settlements; this difference is not significant either.
3. The further investigations of nickle content in biologic material on larger number of examinee from endemic focuses, along with epidemiological study about incidence and prevalence of EN and malignant diseases of urotract in these settlements are needed.
4. The inhabitants of nonendemic rural settlements should be taken in consideration, in order to form a new, specific control group of examinee.

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# BIOMONITORING NIKLA KOD STANOVNIŠTVA U NASELJIMA SA ENDEMSKOM NEFROPATIJOM. PRELIMINARNA STUDIJA

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Endemska nefropatija (EN) je bolest nepoznate etiologije i njena pojava je utvrđena samo na Balkanskom poluostrvu. U okviru ovog istraživanja pošlo se od pretpostavke da nikl može biti faktor rizika ili precipitirajuc'i faktor za nastanak EN i urotelijalnih tumora, s obzirom da predstavlja veoma toksičnu i kancerogenu materiju. Cilj rada je bio da se utvrdi u kojoj meri je nikl, kao kancerogena materija, prisutan u urinu i bubrezima stanovništva sa EN. Kao materijal je korišćeno 93 uzorka urina i 32 uzorka obdukcijskog materijala. Uzroci urina su uzeti metodom slučajnog uzorka od stanovnika endemskih i hipoendemskih sela u slivu Južne Morave i gradskog stanovništva Opštine Niš, kao kontrolnog naselja. Uzorci tkiva bubrega predstavljaju obdukcijski materijal uzet sa leševa ljudi koji su gotovo ceo svoj život proveli u nekom od endemskih ili hipoendemskih sela (ciljna grupa), ili u gradskoj zoni Opštine Niš (kontrolna grupa). Prisustvo nikla odredivano je metodom atomske apsorpcione spektrofotometrije. Koncentracija nikla u urinu veća je kod stanovništva iz endemskih/hipoendcmskih sela nego kod stanovništva kontrolnog naselja, mada razlika nije statistički značajna. Sadržaj nikla u bubrežnom tkivu stanovništva iz endemskih/hipoendemskih žarišta veći je od koncentracije ovog metala u relevantnom autosijskom materijalu stanovnika kontrolnog naselja, ali ni ta razlika nije statistički značajna. Neophodna su dalja ispitivanja sadržaja nikla u biološkom materijalu, paralelno sa epidemiološkim studijama o incidenci i prevalenci EN i malignih oboljenja urotrakta, kod stanovništva endemskih naselja. *Acta Medica Medianae 2003; 42 (3): 15-18.*

***Ključne reči:*** *nikl, urin, bubreg, endemska nefropatija*