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Beryllium concentration in pharyngeal tonsils in children

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Abstract

Power plant dust is believed to be the main source of the increased presence of the element beryllium in the environment which has been detected in the atmospheric air, surface waters, groundwater, soil, food, and cigarette smoke. In humans, beryllium absorption occurs mainly via the respiratory system. The pharyngeal tonsils are located on the roof of the nasopharynx and are in direct contact with dust particles in inhaled air. As a result, the concentration levels of beryllium in the pharyngeal tonsils are likely to be a good indicator of concentration levels in the air. The presented study had two primary aims: to investigate the beryllium concentration in pharyngeal tonsils in children living in southern Poland, and the appropriate reference range for this element in children's pharyngeal tonsils. Pharyngeal tonsils were extracted from a total of 379 children (age 2–17 years, mean 6.2 ± 2.7 years) living in southern Poland. Tonsil samples were mineralized in a closed cycle in a pressure mineralizer PDS 6, using 65% spectrally pure nitric acid. Beryllium concentration was determined using the ICP-AES method with a Perkin Elmer Optima 5300DVTM. The software Statistica v. 9 was used for the statistical analysis. It was found that girls had a significantly greater beryllium concentration in their pharyngeal tonsils than boys. Beryllium concentration varies greatly, mostly according to the place of residence. Based on the study results, the reference value for beryllium in pharyngeal tonsils of children is recommended to be determined at 0.02–0.04 µg/g.

Keywords

pharyngeal tonsil, beryllium, children, southern Poland

INTRODUCTION

Beryllium belongs to a group of trace elements present in very small quantities in the Earth's crust, where the average concentration is around 6 µg/g [1, 2, 3]. It is commonly found in rocks, coals, volcanic dust, and petroleum [2,4]. Beryllium is widely used in the aerospace industry, nuclear energy, and in the military sector because of its ability to increase the hardness and durability of metals [1, 5]. The increasing industrial demand for this element has led to increased production and use, which in turn have led to greater emissions into the environment during extraction and burning processes [4, 6]. Power plant dust is believed to be the main source of the increased presence of beryllium in the environment and it has been detected in the atmospheric air, surface waters, groundwater, soil, food, and cigarette smoke [4, 5, 6, 7]. Beryllium released into the atmosphere can remain present in the air for up to 10 days [4]. In humans, beryllium absorption occurs mainly via the respiratory system, and exposure through breathing results in the accumulation of beryllium in the upper parts of the respiratory tract and the lungs [5, 8, 9].

Beryllium is linked to numerous health problems, and animal studies indicate that this element is a potential inhibitor of various enzymes, including alkaline phosphatase, and can cause disorders in the phosphorescence of proteins crucial to metabolic processes. Exposure can result in general hypersensitivity to this element and autoimmune disease [5]. Beryllium and its compounds disturb the processes of cell growth and cell division, and can interact with DNA to cause genetic mutations, chromosomal aberrations, and DNA breakage [8]. Consequently, beryllium has been found to be a carcinogenic [10, 11, 12]. Exposure to this element results in the increased frequency of malignant lung cancer and osteosarcoma, and may pose a serious threat, especially to people living within reach of industrial emitters (e.g. coal power plants) and municipal dumps [5, 6, 7, 8, 9]. The risk of beryllium exposure is mainly connected with its presence in the inhaled air, although drinking water and food product contamination also pose risks.

Beryllium concentration is typically measured in urine and blood samples [10]. However, in the presented study, measurement of the concentration in the pharyngeal tonsils was selected, primarily because these tissue are located on the roof of the nasopharynx and are in direct contact with dust particles in inhaled air. As a result, the concentration levels of beryllium in the pharyngeal tonsils are likely to be a good indicator of concentration levels in the air. Moreover, because adenoidectomy is a common procedure in children, obtaining tissue samples is relatively easy, thus making it easy to evaluate a large population of children without any extra discomfort and with minimal expense [13, 14, 15]. To our the best of the authors' knowledge, this is the first study to assess beryllium concentration in these glands in children.

Given the important health risks associated with this element, it was decided to perform the study to determine:

1) the beryllium concentration in pharyngeal tonsils in children living in southern Poland;

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2) the appropriate reference range for this element in children's pharyngeal tonsils.

The study was approved by the Bioethical Committee by the Medical University of Silesia in Katowice (Permission No. NN-6501-255/I/04/05).

MATERIALS AND METHOD

Pharyngeal tonsils extracted from a total of 379 children (176 girls, 203 boys) were evaluated in the study. The tonsils had been surgically removed (adenoidectomy) due to hypertrophy in accordance with medical recommendations. The children ages ranged from 2–17 years, with a mean of 6.2 ± 2.7 years (median 6.0 years). The largest group of children were aged 2–6 years and constituted 61%.

All samples were obtained from children living in southern Poland (Tab. 1). The samples of adenoids came from children living in villages located in the Upper Silesian Industrial Region, which is characterized by a high degree of contamination and a large number of industrial centres which are a potential source of berylium emission (R1, R2, R3, R4, R5), from the industrial areas outside the Upper Silesian Industrial Region (R7) and from the agricultural and recreational areas (R6), which are beyond the direct influence of industry.

Table 1. Places where pharyngeal tonsil samples were collected

Place of collecting samples	N	Regions
Zabrze	63	R1
Gliwice,	36	R2
Chorzów, Świętochłowice	66	R3
Tychy	88	R4
Beskid Żywiecki (Żabno, Zator, Rajcza, Węgierska Górka, Żywiec, Polanka Wielka, Wilkowice, Stara Wieś, Pewel ślemieńska, Cięcina, Jawiszowice, Zabrzeg, Lipowa, Kozy, Bystra, Brzeszcze, Bażanowice)	68	R5
Bielsko-Biała	39	R6
Opole	19	R7

Children with a medical history of elemental poisoning, primary haemochromatosis, siderosis, secondary haemochromatosis, and Wilson's disease were excluded from the study.

Prior to analysis, the extracted tonsils were stored in glass vessels in deep-freeze at a temperature of -20 °C. Upon removal from deep-freeze, the samples were defrosted, washed in double-distilled water, and partially dried. The samples were then weighed and brought to constant mass in a KPC-65G drier at a temperature of 105 °C±2 °C. Next, the tonsil samples were mineralized in the closed cycle in a pressure mineralizer PDS 6, using 65% spectrally pure nitric acid.

Beryllium concentration was determined using the ICP-AES method with a Perkin Elmer Optima 5300DVTM, an instrument that allows simultaneous determination of all necessary spectral lines in one run. Berylium was determined using 2 spectral lines that allowed detection of possible spectral interferences. ICP operating parameters were set to robust conditions (i.e. high plasma power, low carrier gas, large injector's internal diameter). Working calibration solutions were prepared from Merck (Darmstadt, Germany) ICP multi-element standard solution VIII. All results were determined as a mean from two replicates recorded in one analytical run. All spectral lines used were verified in terms of number and position of background points and analytical points.

Correctness of the method was checked by means of the method of standard addition (Merck). The limits of detection (LOD) were determined by analysis of method blanks and calculated as 3 times the standard deviation estimate.

The software Statistica v. 9 was used for the statistical analysis. The Shapiro-Wilk test was used to determine normal distribution, while differences between groups were determined using the U Mann-Whitney test for two groups, and the Kruskal-Wallis ANOVA rank test for more than two groups. A p-value of 0.05 was used to determine statistical significance.

Several different methods were used to calculate reference values for beryllium concentration levels. We used the m+ns equation, in which 'm' stands for the average value, n=1 or n=2, and 's' is the standard deviation [16]. The 95 and 97.5 percentiles were calculated, as were the upper limits of the confidence interval [17, 18].

RESULTS

The distribution of beryllium concentration in pharyngeal tonsils in girls and boys is presented in Figures 1 and 2. The distribution was non-normal. The most common finding was a beryllium concentration of ≤ 0.01 μg/g, accounting for 48% and 34%, respectively, of samples taken from boys and girls. In both boys and girls, approximately 30% of the tissue samples had a concentration level ranging from 0.01–0.02 μg/g. A higher concentration range (0.02 μg/g – 0.03 μg/g) was observed in 12% and 16%, respectively, of samples taken from girls and boys. In boys, the highest beryllium concentration level ranged from 0.03 μg/g – 0.05 μg/g, accounting for 10% of the samples. In comparison, the highest range found in girls was from 0.05–0.06 μg/g (4% of samples).

The average overall beryllium concentration in all pharyngeal tonsil sample evaluated was 0.016 μg/g, with

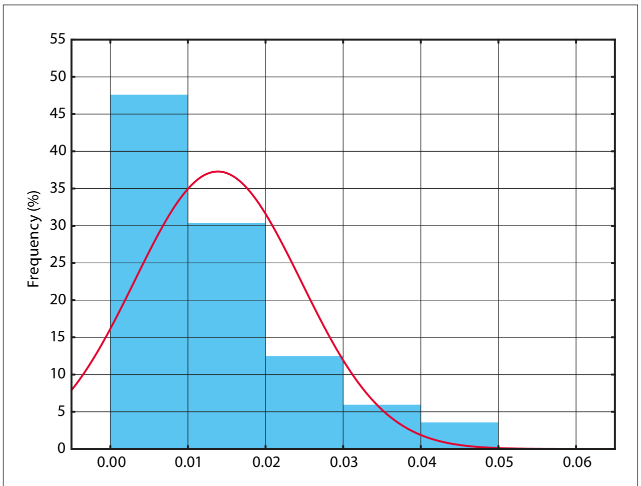

Figure 1. Distribution of beryllium concentration in pharyngeal tonsils in boys

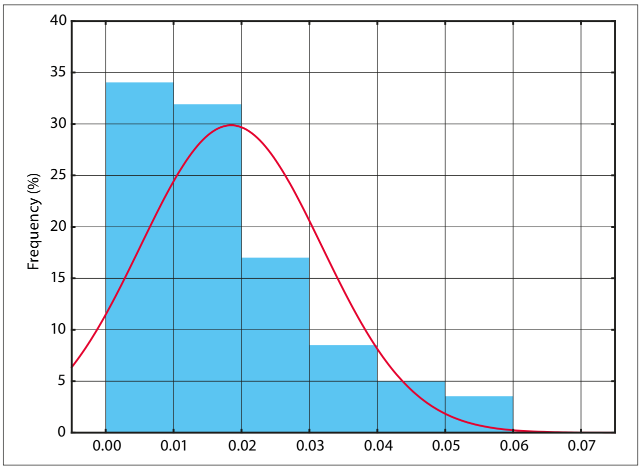

Figure 2. Distribution of beryllium concentration in pharyngeal tonsils in girls

a range of 0.001 µg/g – 0.058 µg/g. The mean beryllium concentration was significantly higher in samples taken from girls (0.017 µg/g vs. 0.014 µg/g) (U Mann-Whitney test p=0.02). (See Table 2 for more details).

Table 2. Beryllium concentration in pharyngeal tonsils in children [µg/g]

Group	No.	Average	SD	Median	Minimum	Maximum	95.0 Percentile	97.5 Percentile	Confidence interval -95.0	Confidence interval +95.0
children	379	0.016	0.012	0.014	0.001	0.058	0.042	0.049	0.011	0.013
boys	203	0.014	0.011	0.011	0.001	0.050	0.036	0.042	0.010	0.012
girls	176	0.017	0.013	0.015	0.002	0.058	0.046	0.051	0.012	0.015

Figure 3 presents the mean beryllium concentration levels by region. The highest concentration levels were found in Zabrze (R1) and Gliwice (R2) (0.025 µg/g). The lowest concentration was found in Bielsko-Biała (R6) and Żywiecki Beskids (R5) (0.010–0.011 µg/g).

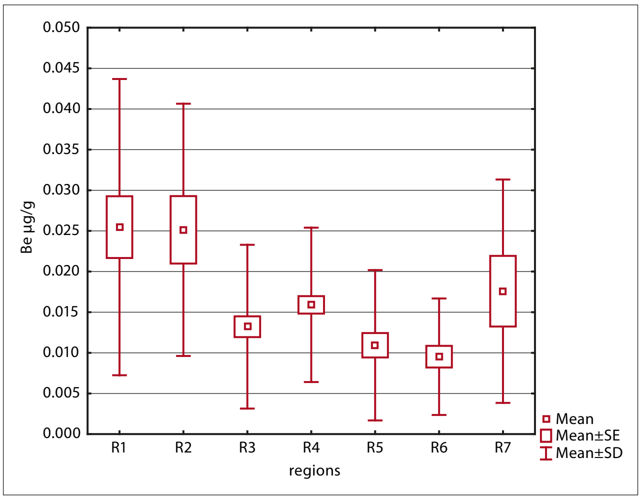

Figure 3. Beryllium concentration in pharyngeal tonsils in children from particular regions of southern Poland

Beryllium concentration levels and ranges by gender and region are presented in Figures 4 and 5. Differences in mean beryllium concentration between regions were statistically

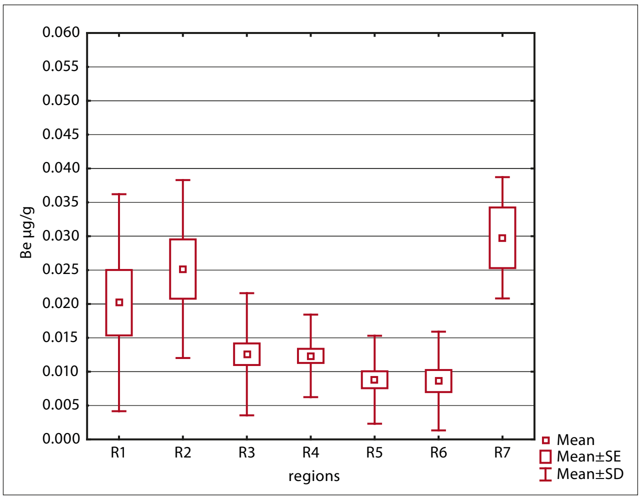

Figure 4. Beryllium concentration in pharyngeal tonsils in boys from particular regions of southern Poland

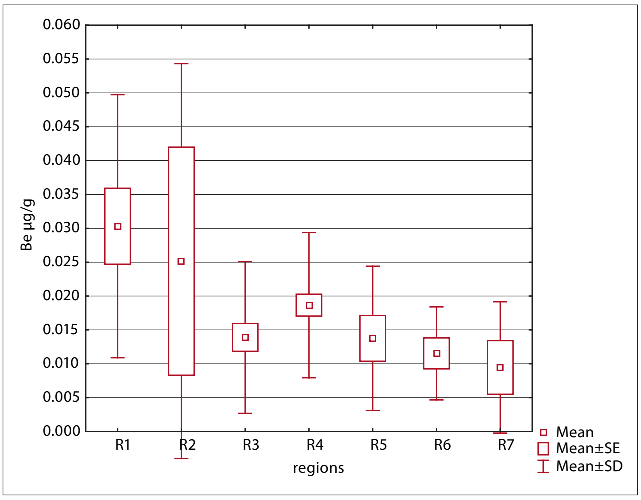

Figure 5. Beryllium concentration in pharyngeal tonsils in girls from particular regions of southern Poland

significant (Kruskal-Wallis test, p=0.00). The highest mean beryllium concentration (0.030 µg/g) was observed in Zabrze (R1) and Opole (R7) for girls and boys, respectively. The lowest mean beryllium concentration (0.009 µg/g) was observed in boys from the regions of Bielsko-Biała (R6) and Żywiecki Beskids (R5), and in girls living in Opole (R7). In Gliwice (R2), the mean beryllium concentration (0.025 µg/g) was the same regardless of gender, although the range for girls (0.002 µg/g to 0.058 µg/g) was greater than that of boys (0.007–0.042 µg/g). In Chorzów and Świętochłowice (R3), the range of beryllium concentration was greater in girls (0.004 µg/g – 0.046 µg/g) than boys (0.003 µg/g – 0.035 µg/g), even though the mean concentration was similar (0.013 µg/g [boys] vs. 0.014 µg/g [girls]). In Tychy (R4), mean concentration levels were considerably higher in girls vs. boys (0.019 µg/g vs. 0.012 µg/g; Mann-Whitney U test, p=0.005), and girls had a significantly greater range of values (0.003–0.051 µg/g) vs. (0.003–0.026 µg/g). In Beskidy (R5), beryllium concentration ranged from 0.001 µg/g – 0.028 µg/g, and boys had a lower mean concentration than girls (0.009 µg/g vs. 0.014 µg/g) (range: 0.002–0.039 µg/g [girls]). Samples from children living in Bielsko-Biała (R6) showed that the range of values was greater for boys

(0.002–0.03 μg/g vs. 0.003–0.023 μg/g), even though the mean beryllium concentration was higher in females (0.009 μg/g vs. 0.0012 μg/g). In the Opole region (R7), the concentration levels ranged from 0.017 μg/g – 0.038 μg/g for boys and from 0.002–0.027 μg/g for girls, and boys had a mean concentration that was 0.021 μg/g higher than girls (U Mann-Whitney test, p=0.025). This difference is more distinct than in other regions and difficult to explain because both girls and boys were at similar age; it seems that the reasons for this difference can be sought in different physical activity for girls and boys.

Values obtained in this way determine the range of beryllium concentration in children's tonsils which can be adopted as the reference value (Fig. 6). The reference value calculated from the m+ns equation (described in Materials and Method) for the arithmetic mean of beryllium concentration in pharyngeal tonsils were as follows: for n=1, the overall mean (all children in the study) was 0.028 μg/g, for girls the mean was 0.030 μg/g (0.002 higher than the overall mean), and for boys – 0.025 (lower than the mean by 0.003 μg/g). For the n=2 calculation, the mean concentration levels were 0.043 μg/g (girls) and 0.036 μg/g (boys); the mean for all children was 0.040 μg/g.

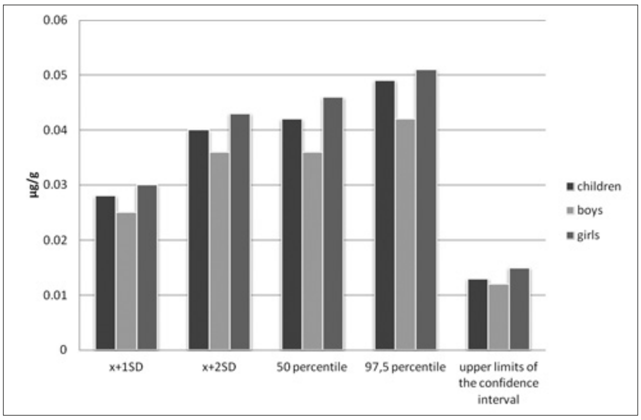

Figure 6. Reference value of beryllium in pharyngeal tonsils in children

Using the 95th percentile as the cut-off point to determine the maximum reference value of beryllium concentration in pharyngeal tonsils in children resulted in the following concentrations: 0.042 μg/g in the overall study population, 0.036 μg/g in boys and 0.046 μg/g in girls. The concentration corresponding to 97.5 percentile was higher in all cases: in girls (0.051 μg/g), in boys (0.042 μg/g) and in the overall study population (0.049 μg/g). The value of the upper confidence interval range around the arithmetic mean was 0.013 μg/g in the whole group, with slightly lower values for boys (0.012 μg/g) and higher ones for girls (0.015 μg/g).

DISCUSSION

Environmental pollution can have a substantial influence on human health. The presence of beryllium in tissues and body fluids may indicate its accumulation in the organism despite excretion processes [4]. It has been hypothesized that increasing environmental levels of beryllium lead to increasing concentration in the organism. The presented study appears to confirm this, as it was found that beryllium concentration in children's pharyngeal tonsils largely depends on where they live. In Poland, the highest mean concentration levels (0.025 μg/g) were found in children living in polluted areas (Regions 1 and 2, Zabrze and Gliwice, respectively) where industrial concentration is the highest. The lowest levels of beryllium concentration, not surprisingly, were found in children living in less polluted areas (Bielsko-Biała [R6], 0.010 μg/g, and Żywiecki Beskids [R5], 0.011 μg/g).

High levels of beryllium concentration in the human body can cause serious health hazards (allergies, cancer) for people living near industrial emitters and municipal dumps [4]. Unfortunately, the growing industrial demand for beryllium seems likely to lead to increasing emissions, which implies potential health hazards for the entire population, particularly children. Children are especially sensitive to the harmful influence of environmental contaminants because their immature immune system cannot fully to protect them from environmental toxins [19].

Beryllium tends to accumulate more in certain human tissues than in others, although some contradictory findings have been reported. According to Kabata-Pendias et al., beryllium concentration in the heart tissue is 0.03 μg/g [20], while Nordberg et al. [21] found levels around 0.07 μg/g (nearly double). The highest beryllium concentration is in the lungs (0.02–0.21 μg/g) [20]. Compared to the findings of the presented study concerning the tonsils, higher mean concentration levels have been reported in the brain (0.08 μg/g), and in the kidneys and spleen (0.07 μg/g) [19]. Other authors have found concentrations in muscles and liver of approximately 0.04 μg/g [20]. Based on the presented data and calculations, the recommended reference value for beryllium in pharyngeal tonsils of children is from 0.02 μg/g – 0.04 μg/g. This range is within the limits of beryllium concentration in soft tissues reported by other authors (0.02 μg/g – 0.21 μg/g) [20,21]. The higher beryllium concentration in pharyngeal tonsils in children compared to the data obtained by Kabata-Pendias can result from the specificity and dynamics of children's behaviour and physical characteristics [20]. Children breathe in more polluted air due to their height (closer to the ground), and have more dynamic vital bodily functions and a faster metabolism than adults. For instance, children have a higher respiratory, caloric, and water/liquids demand, all of which can result in higher beryllium supply to the organism compared to adults [22, 23].

CONCLUSIONS

The presented study shows that girls have a significantly greater beryllium concentration in their pharyngeal tonsils than boys. Beryllium concentration varies greatly, mostly in accordance with the place of residence. Based on the study results, the reference value for beryllium in pharyngeal tonsils of children living in the areas of southern Poland is recommended to be determined at 0.02–0.04 μg/g. Monitoring of these levels to determine population exposure to beryllium is recommended.

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