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ORIGINAL PAPER

FLUORIDE CONTENT IN HAIR IN DEPENDENCE OF THE PLACE OF RESIDENCE, SEX, DIETARY HABITS AND ANTHROPOMETRIC DATA*

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Abstract

Fluoride (F^-) is the most reactive ion present in the human environment. It is commonly known to occur in bones and teeth, and it is used in the prevention of caries. If supplied in excess, fluoride has a toxic effect. In extreme cases, fluoride intoxication may lead to serious illnesses such as the fluorosis of teeth and bones. The aim of the study was to analyse correlations between the content of F^- in hair and environmental factors, nutritional habits and anthropometric data of men and women from various regions of Poland. The level of fluoride was measured in the hair samples from n = 565 people (n = 273 women and n = 292 men, 16.9 to 73.2 years old) using the potentiometric method and an ion-selective electrode. The researchers carried out surveys regarding the personal characteristics of the participants and their nutritional habits. In our study, we demonstrated that despite the use of filters and devices that limit the entry of pollutants into the environment, 15% of the volunteers taking part in our study manifested fluoride values at the same level as employees of an aluminium factory who were exposed to this element due to their place of work. 68% of the examined persons belonged to the category classified as having a moderately increased level of fluoride accumulated in hair. Factors such as

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the gender, size of the village/town and anthropometric conditions did not significantly influence the level of fluoride in hair, unlike the location of one's residence.

Keywords: fluoride, environmental contamination**,** element, BMI, nutrition.

INTRODUCTION

Fluoride (F^-) is a chemical ion commonly present in the human environment. It occurs mainly in air and water (Šket et al. 2017), through which it moves to other trophic levels. Soil includes such minerals as apatite, fluorite, cryolite, secondary clay minerals, aluminum and iron hydroxides, which may release fluoride ions to the environment (Wenzel, Blum 1992, Cronin et al. 2000, Gago et al. 2014). In industrial areas, where the natural environment is more polluted, this process is more intense. A similar correlation is observed in areas of seismic activity (Francisca, Perez 2009). Along with atmospheric precipitation, fluoride finds its way into the soil and groundwater and is later assimilated by plants consumed by humans and animals (including farm animals which serve as food for humans) (Cagetti et al. 2013).

For several decades, scientists have been pointing out the negative effects of fluoride and its compounds on the human organism (Dec et al. 2017, Šket et al. 2017). Fluoride, as the most reactive non-metal, easily reacts with substances that form a living organism. This leads to changes in the activity of enzymes and changes or loss of physiological functions of structural particles present in the human organism. The element is toxic not only in the context of acute intoxications, as they occur relatively rarely. It is dangerous mainly due to the effects caused by long-term exposure to small doses (Choubisa, Choubisa 2016). The negative effects of the activity of fluoride include: an increase in the production of free radicals, the development of an inflammatory state and the negative influence on numerous physiological, biochemical processes (Gutowska et al. 2011). Studies show that chronic, persistent, increased levels of fluoride concentration may lead to chronic kidney diseases (Dharmaratne 2015), the degeneration of bones and teeth – fluorosis (Chakraborti et al. 2016), the damage of the heart muscle, which may negatively influence blood parameters and cause dyslipidemia (Umarani et al. 2015). Fluoride enters the human body mainly through the digestive system with food and water. Studies show that the level of this element in industrial areas is significantly higher (Machoy-Mokrzyńska 2004).

In response to the above information, the following research aim was establish: to analyse the level of fluoride content in the hair of men and women from various regions of Poland. Fluoride level was related to anthropometric data, environmental factors and nutritional habits.

MATERIAL AND METHODS

Patients

Hair samples acquired from volunteers at the Woodstock Festival 2015 in Poland served as the material for labelling the level of F^-. The study was launched as part of a larger project called "Woodstock for Future Health", organised by the employees and students of the Department of Biochemistry and Human Nutrition of the Pomeranian Medical University in Szczecin, Poland.

The calendar age of the volunteers (n = 621) was between 16.9 to 73.2 years old. After signing a consent to take part in the study, the participants completed a survey, in which they provided such information as their age, sex, place of residence, the size of the town/city they lived in and their nutritional habits, i.e. the number of meals consumed per day (no more than 2 meals per day, 3-4/day, ≥5/day), the frequency of vegetable consumption (0-1/day, 2-4/day, ≥5/day), the frequency of fruit consumption (0-1/day, 2-3/day, ≥4/day), the frequency of smoking (every day, from time to time, never), the frequency of alcohol consumption (never, once a month or fewer times a month, 2-4/month, 2-3/week, ≥4/week).

Next, anthropometric data was collected, including: body height (B-v) in cm; body mass in kg; circumferences in cm: waist, hip, shoulder, and the thickness of dermal folds on the shoulder. On the basis of anthropometric data, somatic indexes were calculated – BMI (*Body Mass Index*) and WHR (*Waist-Hip Ratio*). Participants who had dyed hair were excluded from the research. Eventually, the final analysis consisted of n = 565 participants (including n = 273 women and n = 292 men).

The preparation of the samples

The hair was cut with scissors close to the skin of the head, from the occipital side. The hair pieces used as samples were 3-cm long (the length was measured from the skin of the head down). The samples were thoroughly cleansed by washing with appropriate solvents in the following order: distilled water, methanol, distilled water, acetone, distilled water. After drying, the samples were shredded with scissors and 10 mg weight samples were taken from each. The hair was immersed in 2 ml of sodium hydroxide (70% NaOH) for 2 h in 96°C. Subsequently, the samples were cooled down to room temperature, neutralised and replenished with distilled water to 4 ml (OPOROWSKA-MOSZYK, SEŃCZUK 1997).

The labelling of the content of F^- in the prepared samples

The level of fluoride in the hair samples was measured by means of the potentiometric method with the use of an ion-selective electrode (Thermo Orion). To accomplish this, 0.5 ml of a sample, 2 ml of sodium citrate and

2.5 ml of TISAB II were poured to a plastic cup. The measurement of the value of the potential was performed twice: before and after the addition of an appropriate pattern. The acquired results were converted into the level of the content of fluorides in the studied hair samples. Each labelling was performed in three replications.

The statistical analysis and data processing

The data acquired from the measurements of fluoride content were interpreted in line with the work by Kokot and Drzewiecki (2000), who determined the following ranges of values for fluoride content and intoxication: ≤26 mg kg^{-1} F^- – the correct level of fluoride; from >26 to ≤113.7 mg kg^{-1} F^- – moderately increased level of fluoride; over 113.7 mg kg^{-1} F – very high level of fluoride (Kokot, Drzewiecki 2000).

In order to study differences between the groups, the data were submitted to the Kolmogorov-Smirnov test. Because the arrangement of the data deviated from the normal arrangement, nonparametric tests were applied for further statistical analysis: the Mann-Whitney *U* test in the analysis of the variable with a division into two groups, the Kruskal-Wallis test by ranks and the median test (the equivalent of the single factor variance analysis) in the analysis with a division into at least three categories, and the Spearman's rank correlation test.

The level of statistical significance was established at p-value ≤0.05. The statistical analysis was carried out by means of Statistica 12 (StatSoft, Poland). Microsoft Excel 2007 operating on Windows 7 was used to present visually the results of the study.

RESULTS

Descriptive statistics

The mean arithmetic content of fluoride for all of the studied participants was 66.4 ± 63.3 (min 1.7; max 413.1) mg kg^{-1}. Out of all of the analysed cases, according to the criteria established in this study, 68% of the participants had a moderately increased level of fluoride concentration in hair. The "safe", low level of fluoride concentration was observed among 17% of the studied participants. Very high levels of fluoride concentration were observed among the remaining 15%.

The average level of fluoride was $x = 60.88 \pm 56.79$ mg kg^{-1} among women and $x = 71.4 \pm 68.43$ among men. Due to the lack of normal arrangement, the Kolmogorov–Smirnov and the Wald-Wolfowitz tests were used in order to establish if the differences in the mean levels of F among men and women were statistically significant. No significance in terms of differences was observed in any of the two cases (Figure 1).

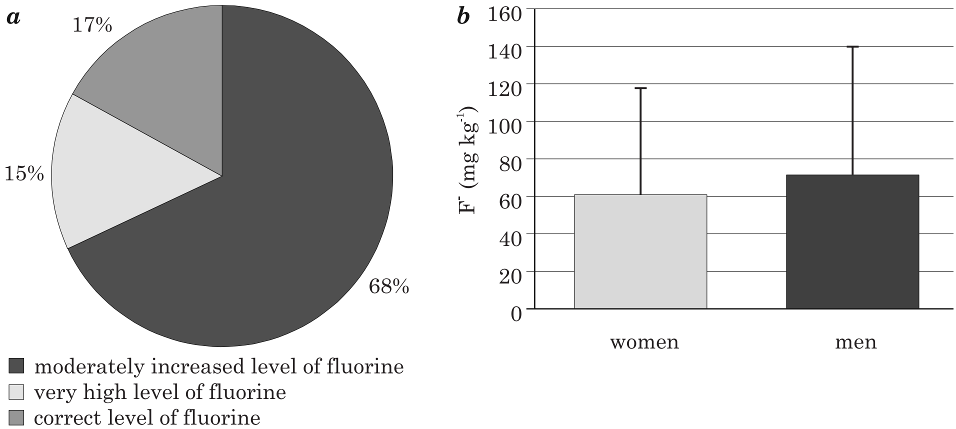

Fig. 1*a*. The exposure to fluoride intoxication determined on the basis of KOKOT, DRZEWIECKI (2000). The accepted levels: from 1 to ≤26 mg kg^{-1} F^{-} – the correct level of fluoride; from >26 to ≤113.7 mg kg^{-1} F^{-} – moderately increased level of fluoride; values above 113.7 mg kg^{-1} F^{-} – very high level of fluoride; *b* – the level of fluoride in hair depending on gender

The level of fluoride in hair in relation to the size of population in one's home town or village

The lowest mean level of fluoride in hair (x = 57.3 ± 41.3 mg kg^{-1}) was observed among people who live in rural areas. On the other hand, the residents of large urban agglomerations (above 200 000 residents) were characterised by the highest mean level of this element (x = 69.7 ± 75.4 mg kg^{-1}). The residents of cities with up to 50 000 population manifested the level of labelled fluoride at x = 68.6 ± 59.0 mg kg^{-1}, whereas the value for medium-sized cities (up to 200 000 residents) was observed to be at the level of x = 62.2 ± 49.4 mg kg^{-1} (Figure 2). The analysis revealed no statistical significance between the content of labelled fluoride in hair and the size of population in the place of residence of the studied subjects.

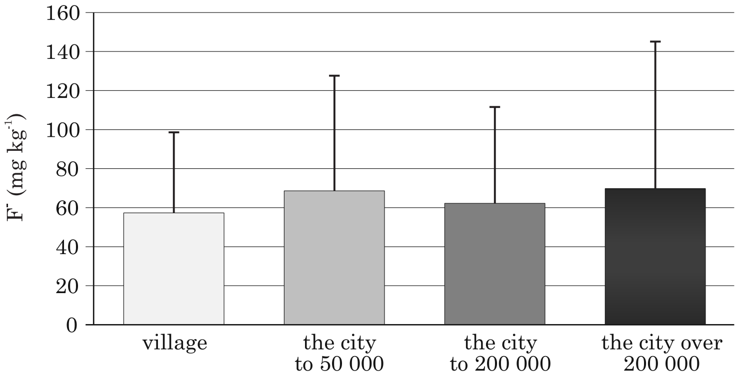

Fig. 2. The level of fluoride in hair divided according to the size of population in the place of residence of research subjects

The level of fluoride in hair in relation to the provinces

The lowest mean level of fluoride labelled in hair samples was observed among the residents of the Świętokrzyskie (*województwo* świętokrzyskie) (x = 38.2 ± 16.99 mg kg^{-1}) and the Province of Lublin (*województwo lubelskie*) (x = 41.1 ± 20.33 mg kg^{-1}). Agriculture of relatively low intensity dominates in both of these regions. The highest mean level of fluoride was observed in provinces with the highest degree of industrialisation: *małopolskie* (x = 82.9 ± 81.07 mg kg^{-1}), śląskie (x = 72.6 ± 59.09 mg kg^{-1}) and *dolnośląskie* (x = 75.3 ± 88.12 mg kg^{-1}). The highest mean levels of fluoride were also observed in provinces with the biggest number of freshwater lakes: *warmińsko--mazurskie* (x = 79.7 ± 71.8 mg kg^{-1}) and *podlaskie* (x = 79.6 ± 80.59 mg kg^{-1}) – Figure 3.

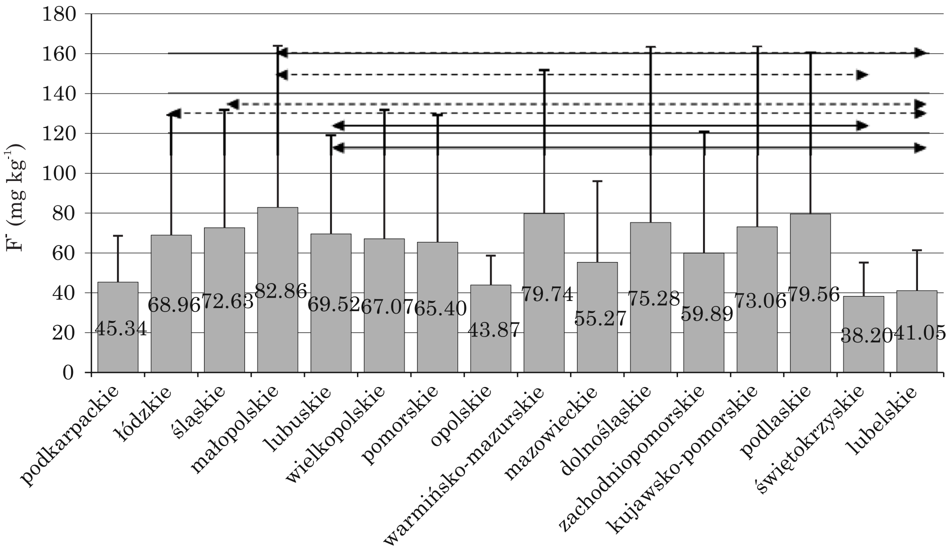

Fig. 3. The level of fluoride labelled in the hair of people who live in specific provinces. The solid line illustrates statistically important differences ($p \leq 0.05$), whereas the dotted line represents differences which are on the threshold of statistical significance ($p \leq 0.1$)

The relation between the fluoride content, anthropometric measurements and somatic indexes

The results were interpreted on the basis of the WHO classification: underweight BMI ≤ 18.49, normal weight BMI 18.50–24.99, overweight BMI ≥ 25.00 <30.00, obese ≥ 30.00 (Ferroluzzi et al. 1995). BMI, commonly referred to as the "nutritional measure", does not characterize the diversity of a diet in terms of its quality and the content of micro- and macronutrients. BMI of the participants was assigned according to the above classification. The analysis of the correlations did not show a significant connection between the concentration of fluoride and their BMI. However, based on the Kruskal-Wallis test, it was established that the level of F^- of the studied women in relation to the groups chosen on the basis of the BMI category differed at statistical significance (H = 8.167126, p = 0.04). The multiple comparisons test (*post-hoc*) showed that the level of F^- (x = 56.95 ± 50.43 mg kg^{-1}) in the hair of women with proper body mass according to BMI (75.5%) differed at statistical significance from the level of F^- (x = 84.35 ± 81.24 mg kg^{-1}) of women with low body mass (8.4%). The F^- level (x = 67.03 ± 68.88 mg kg^{-1}) of overweight women (11.7%) and the F^- level (x = 66.92 ± 64.66 mg kg^{-1}) of obese women (4.4%) in relation to the F^- level of women with proper body mass did not differ at statistical significance. An analogous test carried out on the group of men also did not show statistically significant differences (the value of the Kruskal-Wallis test: H = 3.61726, p = 0.31).

The Waist Hip Ratio (WHR) determines the type of fat tissue distribution in the body. The comparison of the content of F^- in hair of the groups chosen on the basis of the WHR category for the men and women studied (the Mann-Whitney U test) did not show statistically significant differences (men: Z = 0.472929, p < 0.64; women: Z = 0.273727, p < 0.78).

For both men and women, the results of the analysis of correlations of anthropometric measurements vs. the level of F^- were negative or positive with the values close to zero.

The relation between the fluoride content and nutritional habits

The analysis of the Spearman's rank correlation in terms of the relation between F^- content in hair and nutritional habits: the number of meals per day, the frequency of fruit and vegetables' consumption, the frequency of smoking and alcohol consumption in the case of the studied women was not statistically significant in all of the above characteristics. For men, the level of F^- showed a statistically significant relation in terms of the frequency of fruit consumed per day and the frequency of alcohol consumption (respectively: R = 0.1, p = 0.05; R = -0.1, p = 0.03) – Table 1. The Kruskal-Wallis test did

Tab. 1

The relation between fluoride content and nutritional habits

Variables	Women (n = 273)		Men (n = 292)	
	R Spearman	p	R Spearman	p
F (ppm) vs. the number of meals consumed per day	0.1	0.32	-0.1	0.38
F (ppm) vs. the frequency of vegetable consumption	0.1	0.11	**0.1**	**0.05**
F (ppm) vs. the frequency of fruit consumption	0.1	0.06	0.1	0.07
F (ppm) vs. the frequency of smoking	0.15	0.44	-0.02	0.66
F (ppm) vs. the frequency of alcohol consumption	-0.02	0.77	**-0.1**	**0.03**

Statistically significant results given in bold letters.

not show significant differences in the level of F^- between the groups of the studied men chosen on the basis of alcohol consumption frequency. By means of the same test, the differences in the content of F^- were analysed in the groups of men chosen on the basis of the frequency of fruit consumption per day (H = 8.588270, p = 0.01). The multiple comparisons test (*post-hoc*) showed that the level of F^- (x = 73.22 ± 71.10 mg kg^{-1}) in the hair of men who consume fruit 2-3 times per day (43.1%) differed at statistical significance from the level of F^- (x = 66.87 ± 65.95 mg kg^{-1}) of men who do not consume fruit at all, or who eat some only once per day (51.4%). The level of F^- of men who do not consume fruit differed at statistical significance from the level of F^- (x = 101.86 ± 65.58 mg kg^{-1}) of men who declared frequent consumption of fruit (≥4 times per day) – 5.5%.

DISCUSSION

There is a strong correlation between the content of fluoride in urine of people exposed to fluoride and the content of this element in hair. The study results published in 1990 show that the measurement of fluoride in hair can be used as an indicator of fluoride exposure (Czarnowski, Krechniak 1990).

A group of scientists from Gdańsk experimentally checked how fluoride accumulates in the hair of Wistar rats. One subgroup of the studied rats was treated with fluoride supplied with water, whereas the second subgroup received the element via inhalation. Hair samples were taken from the rats every month. After six months, it was established that both methods of fluoride intake resulted in an increase in the concentration of the element in all of the studied body structures of the animals. Moreover, a strong, positive correlation was observed between the content of fluoride in hair and in bones. In the cited study, researchers highlight that hair is an excellent marker of long-term fluoride exposure, whereas the measurements performed on urine and blood may be more significant when studying the effect of short-term exposure (Stolarska et al. 2000). Similarly to hair, nails can serve as study material for determination of the scale of long-term fluoride exposure. The particles of this element are present in nails in the same way as in hair. The research published in 2014 by Indian researchers shows that the fluoride which accumulates in nails is an excellent indicator of the poisoning with this element (Sankhala et al. 2014).

The studies carried out in 1995-1997 on a group of 548 children who lived in the areas of low-risk of fluoride exposure (Ciechanowo, Poland) showed that the average, correct level of fluoride content in hair was from 1.3 to 2.6 mg kg^{-1} (range <0.5 to 26 mg kg^{-1}). In the same research, the level of fluoride was measured among 71 employees who worked under the conditions of high-risk of increased fluoride exposure. The mean values which were labelled in the hair of these people were from 403.4 to 2828.1 mg kg^{-1}, range <113.7 to 5459.8 mg kg^{-1} (Kokot, Drzewiecki 2000). On the basis of these results, it was decided to establish the upper threshold (26 mg kg^{-1}) labelled in the hair of people who are not exposed to fluoride. This maximum value does not indicate intoxication with the element. The values above 113.7 mg kg^{-1}, i.e. the values indicating the lower threshold of the labelled concentration among people strongly exposed to fluoride, were considered in this research as indicative of strong presence of fluoride in the body. The levels of F^- from 26 to 113.7 mg kg^{-1} were considered as intermediate values, which point to an increased risk of fluoride intoxication, but at the same time they do not indicate a very high level of fluoride in the body. In the papers cited herein and in our analyses, the same method of labelling was applied, i.e. an ion-selective electrode measurement.

The present results indicate lack of a significant relation between the gender and the concentration of fluoride in hair in the studied group. The analysis of correlations did not reveal a direct and significant relation between the concentration of fluoride and BMI among the men and women. However, in terms of the same indicator, differences were observed in the comparative analysis of the level of F^- in the scope of four groups (based on BMI) among women. Women with proper body mass (basing on BMI) had the lowest level of F^- in hair. The highest level of F^- in hair was observed among women of low body mass. The Body Mass Index is also referred to as an i ndicator of proper nutrition. It could be presumed that people with proper BMI have a better balanced diet and the quantity and quality of the food they consume is also better. Underweight and excessive body mass may be the results of disorders in the balance of the concentrations of elements in the body and incorrect metabolism (Zofkova et al. 2017).

Among the men, the results indicated differentiated levels of F^- depending on the frequency of consumption of fruit. It is difficult to conclusively associate this data with the level of contamination of fruit and vegetables. Regardless of the place of residence (villages, towns or cities), people buy fruit and vegetables in the same chain stores. However, in smaller communities, the access to local producers who do not use chemical products on a large scale is easier. Based on the survey data, over 44% of the surveyed men lived in rural areas and small towns.

The research published in 2016 by a team of scientists from India showed that among children aged 6-10 there was a small negative correlation between the level of fluoride and BMI (r = -0.083). However, statistical significance was not observed for this correlation (Das, Mondal 2016). Our results and the reviewed papers do not conclusively indicate that body mass is a factor associated with the intoxication with fluoride. Furthermore, the level of F^- does not depend on the type of fat tissue distribution. Labelled according to WHR, the android and gynoid types do not have an influence on the concentration of F^- in the human body.

It is alarming that 15% of the studied volunteers manifested fluoride values at the same level as employees of aluminium works who were exposed to the element due to their occupation (Kokot, Drzewiecki 2000). Moreover, 68% of the studied people belong to the category with a moderately increased level of fluoride accumulated in hair. Interestingly, there is no indication that the residents of rural areas, who are commonly believed to live in places free from toxins, have significantly lower levels of fluoride concentration in hair than do residents of large urban agglomerations. This might be the result of the presence of fluoride in fertilizers used in agriculture (Dey et al. 2012) which, by passing through trophic levels, eventually accumulates in the human body. However, other important sources of fluoride must not be forgotten – water, food and industry (Whitford 1994). The diagnosed situation might also be related to the common use of dental products enriched with fluoride and the process of their production.

Compliance with ethical standards

The authors declare that there are no conflict of interest.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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