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Biomonitoring of Aluminum in Urine of Young Lebanese Children Living in Beirut

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Aluminum (Al) is a ubiquitous, toxic metal to which infants and young toddlers are highly vulnerable. High Al exposure has been associated with various human pathologies. The aim of the present biomonitoring (BM) study was to provide a background for the levels of urinary aluminum (Al) in children ages 7 months to 4 years living in Beirut.

Material/Methods: We collected and analyzed 120 urine specimens using the Shimadzu Atomic Absorption Spectrophotometer-6300 system equipped with an electrothermal atomization, and using a GFA-EX7i graphite furnace.

Results: The mean and standard deviation of Al level in urine revealed 8.978 ± 12.275 $\mu\text{g/L}$, which is within the lower range of each of populations in Germany, Taiwan, and Poland. Vitamin intake, powder rice, and the use of Al utensils proved to be major determinants for Al level in urine (significant at 95%).

Conclusions: The Shimadzu Atomic Absorption Spectrophotometer-6300 system proved again to be an optimal and reliable instrument that can be used for the determination of Al level in urine, especially if using a GFA-EX7i pyrolytic graphite furnace. High levels of Al were found in the urine of Lebanese children. However, the frequent consumption of canned food did not prove to be a significant factor in determining the Al level in urine.

MeSH Keywords: **Aluminum Compounds • Child Health Services • Environmental Monitoring**

Full-text PDF: <https://www.basic.medscimonit.com/abstract/index/idArt/922414>

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Background

Aluminum (Al), the third most abundant element on earth, is a nonessential, toxic metal to which humans are frequently exposed [1]. Al is a ubiquitous chemical that is present in the environment through natural processes (such as soil erosion and volcanic eruptions) and can be found in various products, including pharmaceuticals, cosmetics, and food additives [2–4]. Once in the blood, Al binds to various ligands, with a reported normal human serum concentration equal to 0.04 to 0.12 μM [5,6]. Absorbed Al is excreted principally in the urine, primarily as citrate and, to a lesser extent, in the bile, which constitutes a secondary minor route. Unabsorbed Al is excreted in the feces [7]. Approximately 90% of the excretion of Al by the kidney takes place 48 hours after exposure [8], with a mean urine Al level=0.33 μmole (8.903 $\mu\text{g/L}$) in a typical adult, and mean urinary Al excretion/mole of creatinine=0.77 μmole (20.775 $\mu\text{g/L}$) in full-term infants born at gestation age 39 weeks [5]. Two studies in humans with normal renal function, no specific diet, no medications containing Al, and no other special exposure to Al have reported urine levels of Al of 3.3 $\mu\text{g/L}$ (median) and 8.9 $\mu\text{g/L}$ (mean) [9].

The oral bioavailability of Al from the diet was estimated to be 0.1% to 0.3% in humans based on a normal urinary excretion of 20 to 50 $\mu\text{g Al/person per day}$ and a daily intake of 20 mg Al/person per day [10]. Dietary intakes of 3.5 to 11.5 mg Al/day result in a daily excretion of 4 to 12 μg [8]. In children and young people, the potential estimated exposure at the 97.5th percentile ranged from 0.7 mg/kg bw/week for children aged 3–15 years to 2.3 mg/kg bw/week for toddlers (1.5–4.5 years) in France, and 1.7 mg/kg bw/week for those aged 4–18 years in the UK [9]. In 1993, the FDA has authorized the addition Al to many vaccines as adjuvant to effectively enhance the immune response. Moreover, the Centers for Disease Control and Prevention (CDC) has issued a schedule for vaccines to be given to a child, in which baby, by the age of 18 months, will be injected with 5 mg of Al [11]. A 2-month-old child receiving the recommended vaccinations could receive a minimum potential exposure of 0.295 mg or a maximum potential exposure of 1.2 mg of Al contained within those vaccinations. The potential vaccine dose is well below the minimum risk level (MRL) of 1 mg/kg per day established for Al by the Agency for Toxic Substances and Disease Registry (ATSDR) [12]. In addition, children are exposed to high levels of Al due to the consumption of food that exceeds their body weight [13,14]. Importantly, excess levels of Al have been correlated with various neurological disorders, particularly Alzheimer's disease [6,15], epilepsy [16,17], hypersensitivity reactions [18,19], and bone disorders [20].

Human biomonitoring (HBM) of environmental chemical exposure provides an efficient and cost-effective way to identify and quantify exposures, including those having harmful results on humans [21]. With accumulation of relevant internal

data, HBM can be fully incorporated into risk assessment practices. In addition, the results of HBM form an important basis for future monitoring and research, and for the improvement of guidelines to protect the health of children. This study is highly significant and contributes to HBM studies in Lebanon since infants and young toddlers are highly vulnerable to aluminum. This high exposure is due to children's consumption of food containing high levels of Al and they absorb metals more easily because of their developing endocrine and immature hepatic systems [13,14]. In response to this concern, this study monitored the concentrations of Al among Lebanese children (less than 5 years old) due to the presence of a BM data gap and the high Al exposure of urban children. We also assessed the spread of this metal in Lebanon's environment.

Material and Methods

Instrumentation

Instruments

All the experimental work was carried out on a Shimadzu Atomic Absorption Spectrophotometer (AAS)-6300 230V system, equipped with electrothermal graphite furnace atomization (Shimadzu, EX7i, Tokyo, Japan), an auto-sampler (Model ASC-6100), a deuterium arc background correction system, and a data processing unit. A Hamamatsu Deuterium lamp, a Hamamatsu Al Hollow-Cathode Lamp, a pyrolytically coated graphite tube (64F7280801), a re-circulating water cooler, and a fume extraction system were used. Creatinine measurement was done using a Shimadzu UV-Visible spectrophotometer (Model 2450).

Instrumental conditions

The instrumental parameters used for determination of Al in urine are summarized in Table 1.

Standards and reagents

Standards

A 1000 mg/L Atomic Absorption Al stock reference solution (Sigma-Aldrich) was used to prepare the working standards. Three working standards – 100 000 $\mu\text{g/L}$, 10 000 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ were prepared from a stock solution by 10-fold serial dilution with high-purity deionized water.

Spiked calibration curve matrix-based standards

The working standard, 1000 $\mu\text{g/L}$, was used to prepare 6 spiked calibration standards, ranging from 5 $\mu\text{g/L}$ to 160 $\mu\text{g/L}$, by serial dilution with blank urine for calibration curve construction.

Table 1. Instrumental conditions and parameters.

Type	Model Name	ROM Version	S/N
AA	AA-6300	1.03	A30524100352
ASC	ASC-6000	1.075	A3032410181YS
GFA	GFA-EX7	1.01	A30534100128LP
Furnace Program	Temp.	Time	Flow rate
1	150	20	0.1
2	250	10	0.1
3	1200	10	1
4	1200	10	1
5	1200	3	0
6	2400	3	0
7	2600	2	1
Parameters	Description		
<i>Measurement Parameters</i>	Order: First		
	Zero intercept: Pass		
	Concentration Unit: None		
	Signal processing: Peak area		
<i>Optics Parameters</i>	Element: AL		
	Socket #: 6		
	Lamp current Low (mA): 10		
	Wavelength (nm): 309.3		
	Slit Width (nm): 0.7		
<i>ASC Parameters</i>	Lamp Mode: BGC-D2		
	Rinse Sample: No		
	Mixing: No		
	Injection Volume(μl): 20		
	Injection Speed (μl/sec): 25		
	Intake speed (μl/sec): 130		
Discharge speed (μl/sec): 150			

For preparation of 160 μg/L, 480-μL aliquots from the working standard (1000 μg/L) were transferred to a labeled polypropylene test tube containing 2520 μL of prepared blank urine. Mixing was done by vortexing for 10 s.

Preparations of 80 μg/L, 40 μg/L, 20 μg/L, 10 μg/L, and 5 μg/L were prepared following the same procedure by transferring aliquots of 1500 μL from the previous spiked calibration standard to a labeled polypropylene test tube containing 1500 μL of prepared blank urine. Mixing was performed by vortexing for 10 s.

Reagents

We used argon gas N50, high-purity deionized water, bovine albumin powder (Sigma-Aldrich), creatinine anhydrous (Sigma-Aldrich), potassium chloride (HIMEDIA), sodium chloride (Fluka), sodium phosphate monobasic (Fluka), the SPINREACT Creatinine-J kit, and urea (Sigma-Aldrich).

Controls

Bio-RAD Lyphochek® Heavy metals quality controls for urine of level 1 (20.4–30.6 μg/L) and level 2 (34.2–52.4 μg/L) were

used in this procedure. These 2 controls were used for external quality control. The Al stock standard solution, 1000 µg/L, was diluted with prepared blank urine to prepare the low- (10 µg/L), mid- (20 µg/L), and high-concentration (50 µg/L) internal quality controls.

Blank urine

We dissolved 18.2 g of urea in 750 ml of distilled water in a polypropylene Erlenmeyer flask. Then, 50 mg of bovine albumin powder, 2 g of creatinine, 7.5 g of sodium chloride (monobasic), 4.5 g of potassium chloride, and 4.8 g of sodium phosphate were added and mixed until clear color was obtained. The pH (5–7) of the mixture was checked and then diluted with distilled water to obtain a final volume of 1 L, while placing a hydrometer to check specific gravity, ranging from 1.015 to 1.025. The final mixture was transferred to a plastic storage bottle and stored in the refrigerator at 4°C before use.

Calibration standards

A 1: 1 dilution was prepared for each spiked calibration standard by transferring 500 µL from it to a 2-mL Eppendorf tube containing 500 µL of high-purity deionized water. Mixing was performed by vortexing for 10 s.

Quality control standards

A 1: 1 dilution was prepared for each of the following: Urine Metals Control Level 1, Urine Metals Control Level 2, the low internal quality control (10 µg/L), the mid internal quality control (20 µg/L), and the high internal quality control (50 µg/L) by transferring 500 µL from the designated control solution to a 2-mL Eppendorf tube containing 500 µL of high-purity deionized water. Mixing was performed by vortexing for 10 s.

Sample preparation

A 1: 1 dilution was prepared for each urine sample by transferring 500 µL from the specimen to a 2-mL Eppendorf tube containing 500 µL of high-purity deionized water. Mixing was performed by vortexing for 10 s.

Sampling and specimen collection

Sample size

The target population of this study was healthy boys and girls ages 7 months to 4 years from different regions of Beirut. The total sample size was 120, determined to achieve 90% confidence interval using the Wald method for binomial distribution.

Sample collection

This study included 120 samples were collected from Rasoul Al-Azam's Hospital, 2 pediatric clinics, and 2 day care centers. Ethics approval for conducting the research was obtained throughout. Urine samples were collected after the guardian signed the consent form and filled in a questionnaire (Appendix). The questionnaire included some demographic questions in addition to a few questions about the health status of the child, life style, and parent's knowledge and perception of the toxicants under study. Urine samples were collected in Al-free polyethylene plastic containers (FL Meical®, Italy) without preservatives after careful personal care instructions to avoid dust contamination, and then were transferred to the laboratory. Before analysis, they were stored at 4°C for no longer than 24 h or at –20°C for longer periods.

Analytical procedure

Contamination control

Since Al is ubiquitous, precautions to avoid possible contamination were applied: no glassware was used, high-purity deionized water was used, and a simple dilution procedure with a minimum of specimen handling was developed to prevent contamination through specimen collection, storage, preparation, and measurement. Furthermore, all the standards and controls were prepared fresh on daily basis, and sample dilution was done prior to measurement on a dust-free clean bench. Finally, Aluminum-free propylene plastic containers and plastic labware were used to contribute only negligible amounts of Al to the BM procedure.

Method description

After following the operating instructions for the Shimadzu Atomic Absorption-6300 Spectrophotometer, and setting the optimal instrumental conditions that were previously listed, the stock standards and the working standards were prepared freshly on a daily basis to construct a calibration curve. All the quality controls were diluted prior to measurement and run after the working standards, followed by a batch of 15 freshly diluted duplicated urine samples. The volume of injection was 20 µL. Eight calibration curves were constructed to measure 120 urine samples. This methodology was used due to the availability of valid standard laboratory analytical methods, the feasibility of biological samples collection, the cost of the chemical procedures, and the ethics agreement under national health policies.

Table 2. Calibration curve data.

Conc.	Absorbance						Mean	STD
	1	2	3	4	5	6		
5	0.024	0.0306	0.0156	0.0359	0.025	0.026	0.0262	0.0068
10	0.053	0.0586	0.047	0.056	0.053	0.049	0.0528	0.0043
20	0.082	0.0703	0.0702	0.0796	0.0774	0.077	0.0761	0.0049
40	0.1461	0.1416	0.1454	0.1488	0.1442	0.1434	0.1449	0.0025
80	0.2856	0.2854	0.2854	0.2847	0.2805	0.2766	0.2830	0.0037
160	0.5348	0.5341	0.5328	0.5323	0.533	0.5308	0.5330	0.0014

Creatinine measurement

Urinary creatinine levels were determined by Colorimetric-Kinetic alkaline picrate (Jaffe reaction) using a SPINREACT test kit. Photometric measurements were performed using a Shimadzu UV-Visible spectrophotometer (Model 2450) at 492 nm.

Results

Calibration curve

Data obtained from the calibration curve were linear up to 160 µg/L, which is the maximum anticipated amount of Al to be detected. The curve was the result of a series of 6 injections for each of the following concentrations of Al standard: 5 µg/L, 10 µg/L, 20 µg/L, 40 µg/L, 80 µg/L, and 160 µg/L. The calibration curve was conducted in accordance to the International Conference on Harmonization (ICH), where the linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of analyte in the sample [22]. To do so, absorbance readings at various concentrations, their means, and their standard deviation (STD) are listed in Table 2. As shown in Figure 1, the response (the peak area of standards/peak area of internal standard) is directly proportional to the concentration of the analyte. The correlation coefficient (R) for the analyte was 0.9998, using the equation: $Abs.=0.0032\ Conc.+ 0.0135$

Limit of detection and limit of quantitation

We used the mathematical method for determining the limit of detection (LOD) and the limit of quantitation (LOQ) from the calibration curve (Figure 1). LOD is defined as the lowest analyte in a sample that can be detected, but is not necessarily measured as an exact value. LOQ is defined as the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy [22].

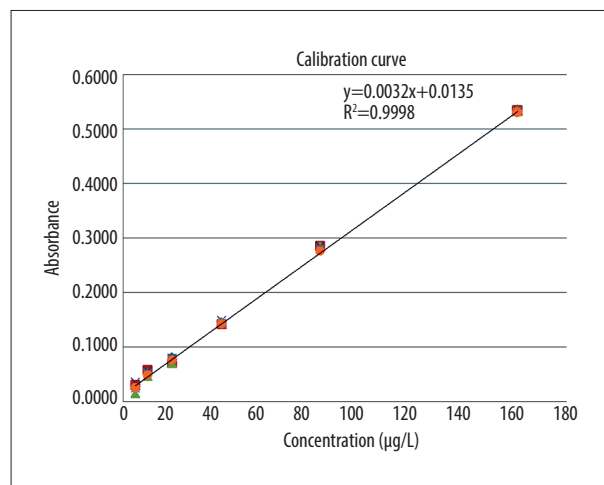


Figure 1. Linear calibration curve for Al.

Several methods are used to detect and quantify limits (LOD and LOQ). The commonly used ones are the visual definition, the signal-to-noise ratio determination, the standard deviation calculation using a linear calibration curve, and the computation using the calibration line at low concentrations. In our study, we used the standard deviation method for a linear calibration curve of the blank model. Under this model, LOD and LOQ can be expressed as:

$$LOD = \frac{3 * S}{b}$$

$$LOQ = \frac{10 * S}{b}$$

where S is the standard deviation and b is the slope of the linear calibration curve of the blank.

The linear calibration curve of the blank is shown in Figure 2, where the correlation coefficient (R) for the analyte was 0.9992, the standard deviation was 0.004127, and the linear equation was: $Abs.=0.0035354\ Conc.+0.0000$.

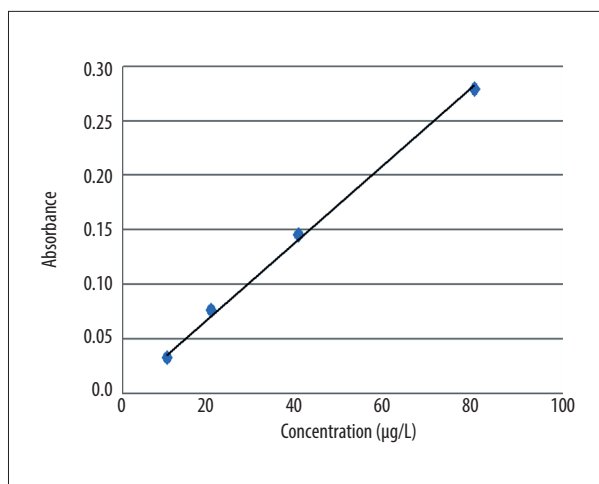


Figure 2. Calibration curve of spiked blank urine.

Using the blank linear calibration curve slope and the standard deviation, results showed the LOD was 3.50201 µg/L and the LOQ was 11.6734 µg/L.

Precision and accuracy

Although accuracy and precision are often used interchangeably, accuracy measures the closeness of a result to a targeted value, while precision shows how close several results are to each other. The overall accuracy was assessed on several different Al levels of urine. This was done at 3 different concentrations: low (10 µg/L), mid (20 µg/L), and high (50 µg/L).

Six injections of each of these 3 concentrations were prepared because repeatability was used as a method for precision. Repeatability indicates precision under the same operating conditions over a short period of time [22].

Table 3 presents the mean, standard deviation (SD), relative standard deviation (RSTD), and accuracy of the 3 different concentrations used. All relative standard deviations (RSTD) were below 5%, which reflected the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling (general precision).

The multi-samples accuracy levels of Al at different concentrations – low (10 µg/L), mid (20 µg/L), and high (50 µg/L) – were determined by dividing each sample Al level by its concentration (Table 4). To determine the multi-samples precision levels, the precision errors were calculated for the 3 concentrations run 6 times using the following equation: % of error=[ABS (mean value–true value)/true value]×100. This equation was selected in accordance with the International Conference on Harmonization (ICH). The precision of an analytical procedure is defined as the closeness of agreement between the values, which is accepted either as a conventional true value or an accepted reference value and the value found [22]. Table 4 presents the results of the 3 concentrations: low (10 µg/L), mid (20 µg/L), and high (50 µg/L), as (1.27%), (3.63%), and (2.65%), respectively. Therefore, a difference of less than 5% of the target value (up or down deviation), at all levels, is classified as very good performance.

Table 3. Precision and accuracy values of Al.

Conc.	1	2	3	4	5	6	Mean	STDEV	RSTD	Accuracy
Low 10	9.6477	9.9067	9.9715	10.0362	9.842	9.9067	9.8851	0.1338	1.35%	98.85%
Mid 20	19.1012	19.1012	20.4609	20.7199	19.1012	19.5221	19.6678	0.7376	3.75%	98.34%
High 50	48.6271	51.8646	51.2171	51.5408	49.0156	49.0156	50.2135	1.4752	2.94%	100.43%

Table 4. Precision and accuracy percentages of Al.

Conc.	1	2	3	4	5	6
Accuracy percentages						
Low 10	96.48%	99.07%	99.72%	100.36%	98.42%	99.07%
Mid 20	95.51%	95.51%	102.30%	103.60%	95.51%	97.61%
High 50	97.25%	103.73%	102.43%	103.08%	98.03%	98.03%
Precision percentages						
Low 10	3.52%	0.93%	0.28%	0.36%	1.58%	0.93%
Mid 20	4.49%	4.49%	2.30%	3.60%	4.49%	2.39%
High 50	2.75%	3.73%	2.43%	3.08%	1.97%	1.97%

Table 5. Quality control results of Al.

Samples	1	2	3	4	5	6	Mean	STDEV	RSTD	Accuracy
Control 1 (25.5 µg/L)	28.1939	23.8808	23.7175	24.6434	25.2976	22.9014	24.7724	1.8659	7.53%	97.15%
Control 2 (43.3 µg/L)	43.2884	46.6464	45.1334	46.3099	45.9456	45.8895	45.5355	1.2115	2.66%	105.16%

Table 6. Frequency of urine Al levels in µmole/L for 120 samples.

Up to	Frequency	Cumulative%	Up to	Frequency	Cumulative%
0.04	38	31.67%	0.38	1	84.17%
0.06	3	34.17%	0.4	2	85.83%
0.08	7	40.00%	0.42	1	86.67%
0.1	8	46.67%	0.44	1	87.50%
0.12	5	50.83%	0.46	1	88.33%
0.14	6	55.83%	0.48	1	89.17%
0.16	6	60.83%	0.52	1	90.00%
0.18	6	65.83%	0.56	1	90.83%
0.2	2	67.50%	0.62	1	91.67%
0.22	4	70.83%	0.74	1	92.50%
0.26	5	75.00%	0.82	1	93.33%
0.3	3	77.50%	0.92	1	94.17%
0.32	3	80.00%	0.94	1	95.00%
0.34	3	82.50%	2	4	98.33%
0.36	1	83.33%	4	1	99.17%
			More	1	100.00%

Quality control

The aim of validation cannot be achieved through ensuring valid analytical data at the beginning of the method only, an should be checked during the entire test. Appropriate control checks should be part of the routine sample analysis. The goal is to optimize the experiment so that, with a minimum number of control analyses, the method and the complete analytical system will provide long-term results to meet the objectives determined in the scope of the method. Quality standards do not require a specific number for the control, but the frequency is expected to be defined, justified, and documented in the procedures [22].

For quality control purposes, the accuracy and precision tests were conducted by injecting 2 control samples (reference-certified material) containing 25.5 µg/L and 43.3 µg/L of Al. These 2 concentrations were injected 6 times, and the results

of the mean, standard deviation (STD), relative standard deviation (RSTD) and accuracy, respectively, are shown in Table 5.

As listed above, the performance score was based on the acceptability criteria of the difference between the obtained result and the targeted value. The mean was within the accepted ranges for both concentrations, 24.77 µg/L ranged between 20.4 and 30.6 µg/L and 45.53 µg/L was between 34.2 and 52.4 µg/L. A difference of less than 10% of the target value, at both 25.5 µg/L (6.37%) and 43.3 µg/L (5.17%), is classified as good performance.

Aluminum levels in urine samples

Table 6 shows the frequency distribution of urine Al values from 120 healthy children. The 90th percentile population showed a urine Al level less than 14 µg/L (0.5 µmole/L). The mean and standard deviation of the urine Al concentration was

Table 7. Significance of coefficients.

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.1981	0.0872	2.2722	0.0254	0.0249	0.3713
Vitamin usage	0.1837	0.0808	2.2730	0.0254	0.0232	0.3442
Canned food	-0.0309	0.0941	-0.3279	0.7437	-0.2178	0.1560
Powder rice	0.3858	0.0912	4.2298	0.0001	0.2046	0.5669
Aluminum utensils	0.2203	0.0925	2.3822	0.0193	0.0366	0.4040

Table 8. Relationship between Al level and common exposures.

	Coefficient	Standard error	t-ratio
Intercept	0.1981	0.0872	*2.2722
Vitamin usage	0.1837	0.0808	*2.2730
Canned food	-0.0309	0.0941	-0.3279
Powder rice	0.3858	0.0912	*4.2298
Aluminum utensils	0.2203	0.0925	*2.3822

Total number of observations=82 urine aluminum detected samples. (*) Means that the variable is statistically significant at the (5%).

8.978±12.275 µg/L (0.333±0.455 µmole/L). Out of 120 samples, the values of Al concentration for 38 samples were unread (ND) since they were below 3.50201 µg/L (0.1297 µmole/L). The nonappearance of Al in a few samples can be a result of exposure at a very early time, permitting the chemical to be eliminated. In addition, the urine Al concentration and standard deviation after creatinine excretion rate in urine for the 82 detected samples was 4.675±22.412 µg/L (done to account for dilution variations). Up to 0.52 µmole/L (14 µg/L) represents 90% of Al levels in samples.

Factors explaining the aluminum levels

Statistical analysis was conducted by regression testing, in which the dependent variable was the level of Al from the tested samples, and 4 main independent variables were used (vitamin intake, canned food, powdered rice, and the use of Al cooking utensils). These independent variables values were extracted from the completed questionnaires. The aim of this test was to identify the main factors determining Al levels in urine.

The results showed that the explanatory power of the estimated models was acceptable since the coefficient of determination was above 50% ($R^2=57.62\%$). Fisher's exact test results led to rejection of the null hypothesis of all coefficients and the significance of the estimates (calculated $F=11.43$).

Table 7 shows there was a significant positive association at the 5% level of significance (95% confidence interval) for vitamin intake, powdered rice, and the use of Al utensils. This

can be explained by the positive signs of 3 coefficients and the P-value less than 5%. Only the intake of canned food did not prove to be a determinant for Al level in urine, since its P-value was higher than 5%. The significance of the 4 independent variables can also be determined from the t test (Table 8).

Discussion

Simultaneous and steady exposure to environmental toxicants, including aluminum, has been associated with critical human health problems. HBM provides an effective technique to better estimate and understand a person's exposure to environmental pollutants [23]. In addition, the results of BM can be used as an important basis for future monitoring and research, and for the improvement of guidelines to protect the health of children [24].

The present study determined Al baseline BM data for young Lebanese children and assessed its spread in the Lebanese environment. This study is based on the understanding that during the last decade there has been a growing concern regarding the heavy and widespread occurrence of Al in the environment. Many empirical studies have revealed the adverse effects of human exposure to Al [5,6], showing that toxic effects can occur at very low doses through accumulation in the human body. Several studies have reported the shortage in data that led to the limit of quantification (LOQ) in the determination of any daily-encountered environmental toxicants. Among these toxicants, Al is considered to be of high concern

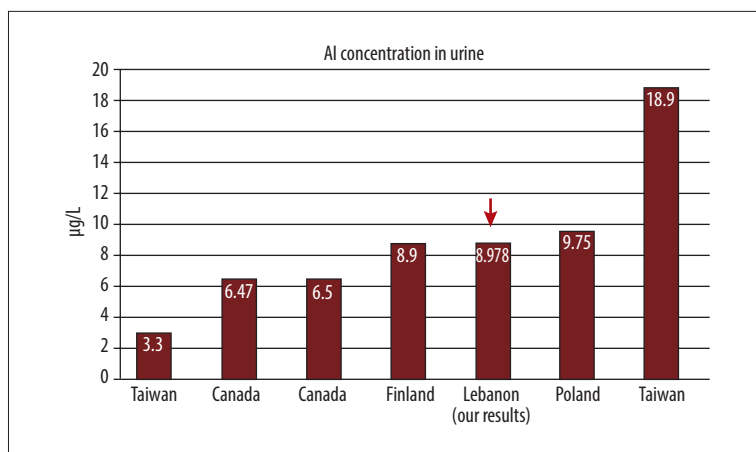


Figure 3. Comparison of Al level with published data.

due to high exposure and potential risk to human health. In this study, we targeted young healthy boys and girls ages 7 months to 4 years from different areas of Beirut for the determination of Al level in urine. The rationale for choosing children less than 5 years old is that they are highly vulnerable to Al due to food consumption that exceeds their body weight, and the robust absorption of metals due to their developing endocrine and hepatic systems. We focused on Al from among all environmental health hazards in children living in Beirut, which is a highly polluted city where urbanization is progressing rapidly, and BM evidence for human exposure to toxicants is incomplete.

For the determination of Al level in urine, a direct method with simple dilution was designed to take a full advantage of the Shimadzu Atomic Absorption Spectrophotometer-6300 230V system. This spectrophotometer included a deuterium arc background correction system and was equipped for electrothermal atomization using a GFA-EX7i pyrolytic graphite furnace. Creatinine measurements were done using a Shimadzu UV-Visible spectrophotometer (Model 2450). The calibration curve is generally constructed either by using the aqueous solution or the matrix-based solution. Since the aqueous solution calibration did not yield appropriate sensitivity, we used the matrix-based solution in determining the Al level in urine. The precision of the method was satisfactory (below 5%). Linearity and accuracy also showed acceptable results. The use of certified reference (control 1 and 2 references) is considered as a key component in the validity of the analytical method. The determined values were within the accepted ranges for both concentrations – 24.77 µg/L was between 20.4 and 30.6 µg/L, and 45.53 µg/L was between 34.2 and 52.4 µg/L. Moreover, there was a difference of less than 10% of the target value, at both 25.5 µg/L (6.37%) and 43.3 µg/L (5.17%) for the control references. The method we used is one of the best procedures for detecting Al level due to its simplicity and simple handling. In addition, there is also less chance for external contamination, it is less time consuming, less labor is involved, and only

20 µg/L of specimen is required with this method. Throughout the study, around 1000 firings were conducted and there were method validation and duplicate firings for samples, with no significant deterioration of the graphite. All specimens were run in duplicate to detect any erroneous result from contamination or graphite deterioration.

The mean and standard deviation of the urine Al concentration in our study was 8.978 ± 12.275 µg/L (0.333 ± 0.455 µmole/L), which is within the lower range of the German population (13–110 µg/L) [25], and also within the lower range of the Taiwan population (2.3–110 µg/L) [26]. It was lower than each of the following reported mean values: 9.75 µg/L in the Poland population [27] and 18.9 µg/L in the Taiwan population [28]. It was higher than each of the following detected mean values: 6.47 µg/L in 63 healthy Canadian adults [29] and 6.5 µg/L in another 28 healthy Canadian adults [30]. Finally, 2 studies were conducted using EFSA; one provided a mean equal to 3.3 µg/L in Taiwan [31], while the other yielded 8.9 µg/L in Finland [32]. As such, there are several ranges for Al level in urine (during 24-h excretion) and our results are in the lower ranges (Figure 3).

While examining the level of Al sample by sample, high levels were observed, which could be due to either the high level of exposure during the last 48 h or high Al gastrointestinal absorption. However, we cannot exclude bias from the use of the first urine void in the morning, which probably has the highest concentration of Al, as well as possible contamination during the process of collection.

While determining the factors that affect Al level in urine, the results showed that the explanatory power of the estimated models was only 57.62%. This result could be explained by the fact that vitamin intake, eating powdered rice, and the use of Al utensils are not the only major sources of Al exposure. Finally, the adopted method has proven to be very reliable, and the Al level in urine for the targeted sample was shown to be acceptable.

Conclusions

The Shimadzu Atomic Absorption Spectrophotometer-6300 system proved again to be an optimal and reliable instrument that can be used for the determination of Al level in urine, especially if using a GFA-EX7i pyrolytic graphite furnace. More importantly, we observed that Lebanese children are exposed to Al and a considerable amount was detected in their urine. Consumption of vitamins and powdered rice and the use of Al utensils are significantly associated with elevated levels of Al

in urine. These factors were expected to influence the Al level in urine since the aforementioned items contain Al. The mean value of urine Al concentration in our study was 8.978 µg/L, which is in the lower accepted range of 20–110 µg/L determined by several studies. Frequent consumption of canned food was not significantly associated with elevated Al levels in urine.

Conflict of interest

None.

Appendix

Products Specification

1. Bovine Serum Albumin – lyophilized powder:

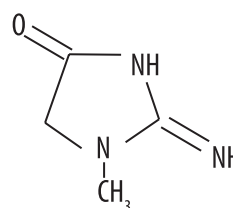
Product Number: A9418
CAS Number: 9048-46-8
MDL: MFCD00130384

Storage Temperature: 2–8°C

TEST	Specification
Appearance (Color)	White to Light Brown
Appearance (Form)	Powder
Solubility (Color)	Faint Green Yellow to Green Yellow to Yellow
Solubility (Turbidity)	Clear to Slightly Hazy
40 mg/mL, H ₂ O	
Loss on Drying	≤5%
Nitrogen	14.5–16.5%
pH	5.0–5.6
(c=1% in H ₂ O)	
UV/VIS Absorbance	≤5
at 406 nm (4% w/v)	
Identity	Bovine Origin
Agarose Electrophoresis	≥96%
% Albumin Cell Culture Test	Pass

2 Creatinine:

Product Number: C4255
CAS Number: 60-27-5
MDL: MFCD00059730
Formula: C₄H₇N₃O
Formula Weight: 113.12 g/mol



TEST	Specification
Appearance (Color)	White to Off White
Appearance (Form)	Powder
Solubility (Color)	Colorless to Faint Yellow

Solubility (Turbidity) 50 mg/mL, H ₂ O	Clear
Loss on Drying	≤1.0%
Carbon	41.6–43.3%
Nitrogen	36.4–37.9%
Infrared	Conforms to Structure
Spectrum Purity (TLC) Suitability	≥98% Suitable
Material tested and found suitable as substrate for creatininase.	
Recommended Retest Period 6 years	-----

3 Potassium Chloride:

Product Number:	P9541
CAS Number:	7447-40-7
MDL:	MFCD00011360
Formula:	KCl
Formula Weight:	74.55 g/mol

TEST	Specification
Appearance (Color)	White
Appearance (Form)	Powder or Crystals
Solubility (Color)	Colorless
Solubility (Turbidity) 200 mg/mL, H ₂ O	Clear
Water (by Karl Fischer) Iron (Fe)	≤2% ≤3 ppm
Heavy Metals (as Lead) DNase, Exonuclease Detection	≤5 ppm None Detected
NICKase, Endonuclease	None Detected
Detection RNase Detection	None Detected
Protease Detection – FITC	None Detected
Titration by AgNO ₃ Based on chloride content	None Detected ≥99.0%
Recommended Retest Period 4 years	-----

4 Sodium Chloride:

Product Number:	S9888
CAS Number:	7647-14-5
MDL:	MFCD00003477
Formula:	ClNa
Formula Weight:	58.44 g/mol

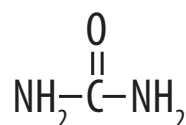
TEST	Specification
Appearance (Color)	White
Appearance (Form)	Powder
Meets ACS Requirements	-----
Titration with AgNO ₃	99.0–101.5%
Insoluble matter	≤0.005%
pH	5.0–9.0

5 % solution at 25 deg C

Iodide	≤0.002%
Bromide	≤0.01%
Chlorate and Nitrate (as NO ₃)	≤0.003%
Phosphate	≤5 ppm
Sulfate	≤0.004%
Barium	Pass
Calcium (Ca)	≤0.002%
Magnesium (Mg)	≤0.001%
Iron (Fe)	≤2 ppm
Potassium (K)	≤0.005%
Heavy Metals	≤5 ppm

5 Urea:

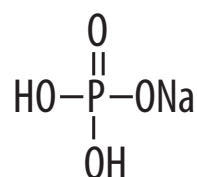
Product Number:	U4884
CAS Number:	57-13-6
MDL:	MFCD00008022
Formula:	CH ₄ N ₂ O
Formula Weight:	60.06 g/mol



TEST	Specification
Identity	Pass
Residue on ignition (Ash)	≤0.1%
Insoluble matter	≤0.04%
Alcohol-insoluble matter Assay	
Organic Impurities	98.0–102.0%
Residual Solvents Recommended Retest	Pass
Period 5 years	Meets Requirements

6 Sodium Phosphate Monobasic:

Product Number:	S2554
CAS Number:	7558-80-7
MDL:	MFCD00003527
Formula:	H ₂ NaO ₄ P
Formula Weight:	119.98 g/mol



TEST	Specification
Identity	Pass
pH	4.1–4.5
5% Solution	
Water Content by Karl Fischer	Pass
<2.0%	
Insoluble Substance	≤0.2 %
Chloride	Pass
< or =0.014%	
Sulfate	Pass
< or =0.15%	
Aluminum Ca & Related Elements	Pass
Arsenic (As)	Pass

≤8 ppm

Heavy Metals

< or =0.002%

Residual Solvent s USP 467

Assay

Anhydrous Basis Recommended

Retest Period 4 Years

Pass

Meets Requirements

98.0–103.0%

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