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Is There Correlation Between Aluminum-Based Food Consumption and Plasma Level in Pregnant Women?

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Abstract
To explore the correlation of AL-based food consumption, known to have negative impact on health, and Al plasma levels with pregnancy status. A cross-sectional study was conducted on 75 participants, including 50 pregnant women. Al plasma levels were analyzed by ET-AAS. Exposure to food was positively correlated to Al mean plasma levels (reaching 2.12 ± 1.17 µg/L) by 32%, specifically for potatoes, fruits, soft drinks, and ready meals. Usage of Al cookware was associated to higher Al plasma levels while pregnancy status was protective. Establishment of national recommendation to maintain lower levels of Al in food is required.

Keywords Aluminum · ET-AAS · Exposure · Plasma level · Pregnant · Lebanon

Introduction

Exposure to aluminum (Al) from food stems from various different sources. Al is naturally found in the soil and therefore is absorbed by plants and subsequently consumed by humans [1]. Al also enters our foods through additives, food packaging, and utensils used in food preparation [2, 3]. Al was long been considered to be harmless to humans, due in particular to its very low intestinal absorption by the oral route. However, research has shown that Al can be toxic to the human body once it begins to alter the intestinal wall, following cell apoptosis and oxidative stress [4]. As precursor of neurodegenerative diseases initiated and enhanced by oxidative stress due to Al interference, Al induces neuronal apoptosis and stimulates active microglia by the mean of an immune and excitotoxicity process, releasing therefore pro-inflammatory cytokine/chemokine and excitatory amino acids, particularly glutamate that cause damage to neurons [5, 6]. According to previous studies, the exposure to metals (including Al) has adverse effects on children's health, including lower birth weight, anogenital distance, Apgar scores, lung function, hepatitis B surface antibody levels, higher prevalence of attention-deficit/hyperactivity disorder, and DNA and chromosome damage [7, 8]. Oral Al exposure during pregnancy has been shown to have a significant effect on tissue distribution for a number of essential elements

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[9]. Recent studies revealed that Al and Al/Mg levels had a positive association with inflammatory mRNA expression and placental oxidative stress in the second trimester of pregnancy [10]; oxidative stress is the cause of pregnancy-related diseases such as embryo absorption, pre-eclampsia, and intrauterine growth restriction [11] as well as placental inflammation [12]. Another study showed a positive correlation with Al plasma level and the risk of having gestational diabetes (GDM) through the mediation of plasma polyunsaturated fatty acids (n-6 PUFAs) [13]. Normal Al plasma levels are known to be less than 10 µg/L; toxicity was associated with higher plasma levels of Al (above 100 µg/L), with clinical symptoms of toxicity typically manifesting at levels above 200 µg/L [14]. Many age groups are sensitive to these health parameters, specifically women of childbearing age and elderly individuals. The proven clinical effects of Al have been observed in situations of high chronic exposure, but no study into the general population's exposure through current diet or health products has ever demonstrated such effects. Our objective is first to study the relation between Al-based food consumption comparing plasma blood levels of Al in pregnant vs. non-pregnant women, and to identify any association between behavioral factors and higher exposure.

Material and Methods

Recruitment of Participants and Study Design

A secondary data from a cross-sectional study [15] using a self-administered e-FFQ was collected to evaluate Al plasma level of female participants between the ages of 18 and 47, after having their consent. The recruitment was done randomly over a period of 1 month (August 2019) at Rafic Hariri University Hospital (RHUH), located in the capital Beirut, subsequently to having obtained the approval of the hospital's Institutional Review Board (IRB) on the study protocol, questionnaire, and consent form. RHUH IRB agreed with the regulatory requirements of the Food and Drug Administration (FDA) and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The sample size was calculated by a previous pilot study done on 12 participants, based on an Al plasma mean of 0.255 µg/L and using the following formula with mean error of 10%, $n = (1.96*0.255/0.075)$ square = 44.40 if E10% of mean. The required sample size was 50. Total sum of 75 female participants including 50 pregnant women was then considered in this observational study.

Data Collection and e-FFQ

The questionnaire entailed questions on socio-demographics, lifestyle (presence of specific diseases, consumption of specific medication), habits related to Al use (mainly usage of Al foil and Al utensils while cooking), and food frequency consumption. Exclusion criteria were limited to chronic renal failure and/or hemodialysis procedure. A customized web-based platform registered under Curve® was used after being pilot tested to collect data and ease analysis [15]. For the estimation of daily dietary exposure (DDE) of Al for each food item, we have multiplied the concentration of Al in selected food items (mg/kg) with their mean consumption per day retrieved from the results of E-FFQ with the weight of each portion size (g). The sum of all items ended with the below DDE using the equation: *Daily dietary exposure (mg/day) = Σ [element food content (mg/kg) × food intake (g/day)] /1000.*

For more details, refer to article [15].

Collection and Transfer of Blood Samples

Blood samples were collected by the hospital laboratory department phlebotomists. After following the study protocol and, to ensure a contamination free and quality of sampling, manipulation and precaution standards were followed. During skin disinfection, chlorine-based antiseptic was used (Dakin®), blood was collected in specific trace elements tubes (6 mL, polyethylene terephthalate (PET) Vacumed® tubes containing K2 EDTA (anticoagulant), Ref 43,016—FL Medical), and stainless steel needles with Al-free flaps using vacutainer closed system and nitrile gloves (free of powder) were used during the withdrawal of blood. All collection instruments were free of any Al traces. After blood collection, the samples were kept in a cooler at a temperature of 4 °C, and were transferred during the same day to the American University of Science and Technology-Analytical Testing Laboratories (AUST-ATL). They were kept at the same temperature until they were processed in the laboratory.

Sample Preparation and Matrix Modifier

Sample preparation involved (1) rinsing all used vials, cones, and tips with 10% hydrochloric acid (HCl) followed by 10% nitric acid (HNO_3); (2) centrifuging blood samples for 5 min and 2565 RCF using Heraeus Multifuge 1S-R Centrifuge; (3) transferring plasma to another trace element tube (same reference as above) using a micropipette after decantation; and (4) storing samples at a temperature of 4 °C until analysis. A matrix modifier composed of 10 mL of phosphoric acid stock (H_3PO_4) [20624.420 — VWR], 1 mL palladium [76040 — Sigma-Aldrich], 10 mL Triton X-100 [T8787 — Sigma-Aldrich], and 10 mL of HNO_3 [20429.320 — VWR] was used to minimize the influence of interference due to the matrix, and to promote stabilization of the oven's inner temperature by increasing the pyrolysis temperature and thus reducing the effects on the matrix. The volume was

completed up to 1000 mL with deionized water. In addition, this procedure improves the signal separation from the analyte and background noise.

Metal Analysis in Plasma and Quality Assurance

Al measurement was performed on an Electrothermal Atomic Absorption Spectrophotometry (ET-AAS) model AA type 6300/GFA-EX7i, equipped with an ASC-6100 auto-sampler (Shimadzu). The wavelength of the hollow cathode lamp was 309.3 nm and the slit width was 0.7 nm. The operating parameters for the working element were set as recommended by the manufacturer on 8 steps heat (RAMP, RAMP, STEP, RAMP, STEP, STEP, STEP, STEP), temperature in °C (80, 120, 120, 1400, 1400, 1400, 2400, 2700), and Ramp time in seconds (20, 10, 12, 10, 9, 3, 3, 2) with internal N_2 flow L/min (0.10, 0.10, 1.00, 1.00, 1.00, 0.00, 0.00, 1.00).

For curve calibration, the method of standard addition was used. Starting from an Al stock solution of 1000 ppb, 5 different concentrations were prepared in vials (5, 2.5, 1.25, 0.625, and 0.3125 ppb), and 250 µL of matrix modifier was added to each vial after they were diluted ½ in series from the vial of 5 ppb, to reach 0.3125 ppb after adding 250 µL of Al stock to the vial of 5 ppb. The samples were then prepared from an initial volume of 400 µL (100 µL of the plasma and 300 µL of matrix modifier) with stirring. The following statistical characteristics were determined based on the standard deviation of several blanks: limit of detection 0.33 ppb; limit of quantification 0.53 ppb. Repeatability was calculated to represent an uncertainty of measurement for low concentration of 0.625 ppb (Bias % − 2.54, RSD % 9.26) and for high concentration of 5 ppb (Bias % 0.09, RSD % 0.43). Curve metrics are shown in Fig. 1.

Statistical Analysis

Statistical analysis was carried out using IBM SPSS software version 25. The data was presented by mean and standard deviation. A *p*-value of less than 0.05 was considered significant. To study the correlation with selected food items and Al plasma levels, Pearson correlation was established, and Spearman correlation was used when number of participants was relatively small. In addition, independent Student's tests were performed for the association of pregnancy status and lifestyle with Al plasma levels.

Results

Al plasma mean for the total participants was 2.12 ± 1.17 µg/L. A significant difference of Al plasma levels was found with regarding pregnancy status ($p < 0.023$). Pregnant women had lower Al plasma mean than non-pregnant women (1.90 ± 1.02 µg/L [CI 95% 1.61–2.19] vs

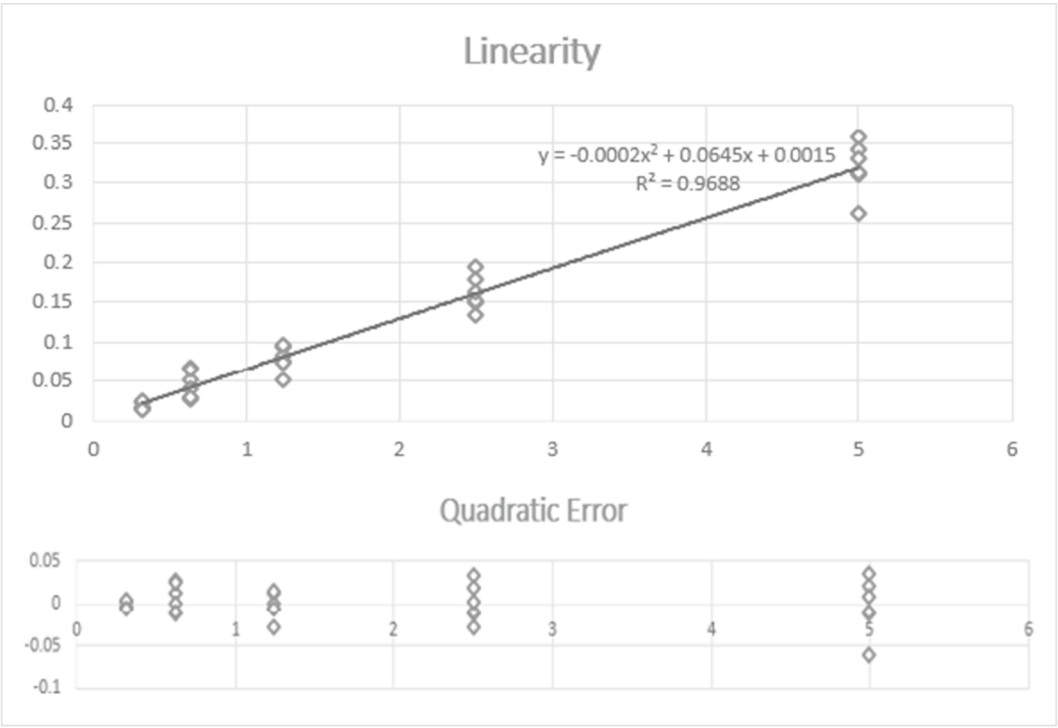

Fig. 1 Curve metrics and lack-of-fit quadratic error

2.55 ± 1.35 µg/L, $p < 0.005$ [CI 95% 1.99–3.11]) ranging from 0.33 to 4.36 µg/L and 0.40–5.23 µg/L respectively. There were no significant difference seen for the pregnancy status and the use of Al products with food (e.g., Al foil and others) (73% vs. 60.5%; $p < 0.253$), as well as for their use of Al-based utensils while cooking (73.1% vs. 52.2%; $p < 0.077$). We have found a significant difference between participants who use Al utensils in cooking and those who do not, considering Al plasma mean (2.51 ± 1.29 µg/L [95% CI 2.01–3.01] vs. 1.88 ± 1.05 µg/L [95% CI 1.57–2.19]; $p < 0.024$).

Participants demonstrated positive correlations between food exposure rates and plasma Al levels by 32%, specifically in potatoes, fruits, soft drinks, and ready meals. Al plasma levels increase with the consumption of food as follows: potatoes 35% ($p < 0.002$), fruits 33% ($p < 0.003$), soft drinks 25% ($p < 0.029$), ready meals 24% ($p < 0.035$). Being pregnant had only positive correlation with the consumption of sauce and condiments by ~ 31%. When excluding pregnant participants, we had higher positive correlation for the overall food exposure by 47% ($p < 0.021$), mainly for ready meals 47% ($p < 0.019$), fruits 46% ($p < 0.015$), and juices 41% ($p < 0.040$) (Table 1).

To seek the difference between food consumption and the exposure of pregnant and non-pregnant women, we have tested in Table 2 the association of food exposure with pregnancy status, which shows the following results for the total food exposure (102 for the pregnant vs. 113 for non-pregnant; $p < 0.234$). The presence of a significant difference in food consumption was found in the dessert group where pregnant women had twice the consumption of desserts, cream, and jams (mean exposure of 7.33 for pregnant vs. 4.54 for non-pregnant, $p < 0.03426$), and less exposure (approximately 3 times less) to candies than non-pregnant women (0.21 for pregnant vs. 0.61 for non-pregnant, $p < 0.014$) and (approximately by two-third) to ready meals (4.28 for pregnant vs. 5.98 for non-pregnant, $p < 0.023$ (Table 2).

For the effect of age on the correlation between dietary and plasma Al, we have found a significant positive correlation in the group age of 25–35 years with their daily exposure to fruits (33%), soft drinks (32%), potatoes (27%), and processed cheese (26%). Thus, after being segregated by pregnancy status, the correlation was significantly positive in pregnant women with their daily exposure to fruits by (35%) and in non-pregnant women with their exposure to soft drinks by (55%).

Discussion

Pregnancy status was not associated with Al plasma level for the total exposure to Al-based consumed food; pregnant women have lower Al plasma levels than non-pregnant

Table 1 Correlation of Al plasma level with the exposure to consumed food

Food categories	Al-based food exposure	Al in plasma Total sample ($N = 75$)	p value[+]	Al in plasma Pregnant ($N = 50$)	p value[+]	Al in plasma None pregnant ($N = 25$)	p value[‡]
Total exposure to food		0.323*	0.005	0.155	0.283	0.467*	0.021
Vegetables and fruit	Fruits	0.333*	0.003	0.202	0.160	0.464*	0.015
	Vegetables	0.052	0.656	0.046	0.751	0.082	0.697
	Potatoes	0.350*	0.002	0.025	0.862	0.355	0.082
	Legumes	0.009	0.938	0.036	0.805	0.061	0.779
Bread and pastry	Bread and pastry	0.128	0.273	− 0.034	0.816	0.128	0.542
	Cake, croissant, and biscuit	0.188	0.106	− 0.033	0.819	0.261	0.207
Dairy	Dairy products	0.094	0.427	0.023	0.874	0.388	0.061
	Processed cheese	0.198	0.089	0.264	0.063	0.082	0.696
Meats	Chicken fish meat	0.155	0.184	0.135	0.350	0.061	0.771
	Charcuterie	0.122	0.299	− 0.210	0.143	0.284	0.169
Beverages	Coffee cocoa tea	− 0.011	0.923	0.165	0.252	− 0.058	0.781
	Juices	0.160	0.171	− 0.061	0.676	0.414*	0.040
	Soft drinks	0.253*	0.029	0.139	0.336	0.333	0.104
Dessert	Arabic sweet	0.081	0.209	0.113	0.436	0.256	0.227
	Dessert and cream jam	− 0.013	0.909	0.019	0.897	0.096	0.647
	Candies	0.221	0.057	0.215	0.133	0.052	0.805
Fast food and ready sauces	Ready meals	0.246*	0.035	− 0.023	0.875	0.475*	0.019
	Sauce and condiments	0.056	0.634	0.308*	0.030	− 0.175	0.404

[+]Pearson correlation; [‡]Spearman correlation; *Significant correlation

Table 2 Mean exposures to Al-based food groups and pregnancy status

Exposure to Al-based food groups		Pregnancy status (non-pregnant vs. pregnant)			
		Mean (mg/day)	95% CI bound	Min–max	*p* value*
Total food exposure		113.29 ± 51.38 101.86 ± 30.40	[91.60–134.99] [93.22–110.50]	43.30–237.01 42.01–190.18	0.234
Vegetables and fruit	Fruits	4.38 ± 4.62 2.74 ± 2.02	[2.47–6.29] [2.17–3.32]	0.00–17.50 0.00–7.00	0.035
	Vegetables	25.21 ± 16.22 24.61 ± 7.85	[18.51–31.90] [22.38–26.84]	1.37–60.71 10.44–65.15	0.831
	Potatoes	6.98 ± 8.36 4.13 ± 2.79	[3.53–10.43] [3.34–4.93]	0.20–34.67 0.00–13.08	0.032
	Legumes	7.17 ± 7.58 6.69 ± 2.82	[3.96–10.37] [5.88–7.49]	0.00–37.71 0.00–10.06	0.694
Bread and pastry	Bread and pastry	7.26 ± 5.05 6.76 ± 3.77	[5.17–9.35] [5.68–7.83]	1.82–21.84 0.00–21.49	0.631
	Cake, croissant, and biscuits	2.84 ± 3.29 2.87 ± 1.68	[1.48–4.20] [2.39–3.35]	0.00–10.36 0.00–6.19	0.963
Dairy	Dairy products	6.08 ± 4.71 7.62 ± 6.33	[4.09–8.07] [5.82–9.42]	0.73–14.54 0.00–33.77	0.295
	Processed cheese	4.11 ± 4.61 3.82 ± 3.47	[2.20–6.01] [2.83–4.80]	0.00–16.43 0.00–16.43	0.760
Meats	Chicken, fish, and meat	3.82 ± 3.38 3.81 ± 2.83	[2.42–5.22] [3.01–4.62]	0.00–14.90 0.20–11.52	0.993
	Charcuterie	0.71 ± 1.65 0.29 ± 0.96	[0.03–1.40] [0.02–0.57]	0.00–7.68 0.00–4.61	0.169
Beverages	Coffee, cocoa, and tea	11.54 ± 10.72 8.61 ± 6.45	[7.11–15.97] [6.78–10.45]	0.58–51.49 0.00–26.95	0.145
	Juices	6.35 ± 6.81 5.29 ± 6.34	[3.54–9.16] [3.49–7.09]	0.00–19.10 0.00–20.00	0.507
	Soft drinks	10.30 ± 12.51 8.15 ± 8.01	[5.13–15.46] [5.87–10.43]	0.00–53.00 0.00–21.20	0.371
Dessert	Arabic sweet	0.56 ± 0.71 0.82 ± 1.35	[0.26–0.86] [0.44–1.21]	0.00–3.39 0.00–5.58	0.373
	Dessert, cream, and jam	4.54 ± 5.48 7.33 ± 4.72	[2.28–6.81] [5.98–8.67]	0.00–25.42 0.00–25.54	0.026
	Candies	0.61 ± 0.70 0.21 ± 0.63	[0.32–0.90] [0.03–0.39]	0.00–2.53 0.00–2.53	0.014
Fast food and ready sauces	Ready meals	5.98 ± 3.92 4.28 ± 2.36	[4.32–7.64] [3.60–4.95]	2.04–17.32 2.11–10.18	0.023
	Sauce and condiments	3.25 ± 2.42 3.75 ± 1.89	[2.25–4.25] [3.21–4.29]	0.22–9.55 0.00–7.44	0.336

*T-test

women regardless of food exposure types with a mean difference of 0.65 µg/L, $p < 0.023$, which might have a protective aspect in a way or other. In a prenatal study correlating in utero exposure to Al in pregnant women and its status on delivery, the outcome highlighted that serum Al and urine Al levels were not correlated; therefore, Al retention would have occurred during pregnancy [16]. Mothers who ate root vegetables during pregnancy were less likely to have high maternal serum Al levels compared to others. The study did not find any significant correlation between serum Al concentrations and other dietary intakes, including beverages. Unlike our own study, mean plasma Al levels were positively correlated to sauce and condiments by 31% (Table 1). Since pregnant women typically had lower Al plasma means to food exposure compared to non-pregnant women, this might be due to the presence of hemodilution during pregnancy or it might lead to the retention of Al in the body and the increase of tissue concentration. Therefore, fetuses may be predisposed towards Al exposure through the placental path, as noted in studies [17, 18], or might have a protective predisposition to Al circulatory absorption which might be due to the changes in food habits during pregnancy. Nevertheless,

circulating Al levels were within the normal range in all groups regardless of their correlation with food intake.

Al is found in many different types of foods and beverages. The major source of dietary exposure is typically found in fruits and vegetables. However, we cannot exclude the potential Al exposure that occurs through beverages, especially soft drinks, its presence in bread and pastry, and ready meals, especially through Al trays, Al foil, and other materials [19]. Al utensils are frequently used in private households, such as drink containers, coffee pots, grill pans, and camping cookware, which can lead to Al contamination as metal leaches into the food during storage or preparation [20]. Consequently, individuals should control their dietary intake by limiting their use of Al utensils in cooking and Al-based food additives, as well as limiting their consumption of beverages that come kept in Al containers, such as soft drinks. Regulatory authorities should investigate and monitor the presence of Al in both soil irrigation water and drinking water (in Lebanon studies have only focused on water quality and other trace elements). Additionally, they should set guidelines for the food market regarding the preparation of ready meals, as well as the proper use of Al wares and packaging. As Al in known to be absorbed and detected in blood [5], our results showed a positive correlation between Al food intake and plasma Al levels, ranging from 24 to ~ 47%. Therefore, we cannot exclude the predisposition of the studied sample to have cumulative Al depositing in their organ tissues and hence their future exposure to emergent related diseases. Vigilance must be maintained in all areas of Lebanon to check the quality of drinking water, as well as food, to maintain a low level of Al impregnation as this study was limited to food retrieved from Beirut market.

Conclusion

This is the first study to evaluate the Al mean plasma level in Lebanese pregnant women and to reveal its association with food exposure. The low Al plasma mean for all participants was 2.12 µg/L, and our results showed similarity to those reported by other studies for normal individuals with less than 10 µg/L that these levels are considered safe and likely of no clinical significance. This would place, as a first attempt, the Lebanese population behind in terms of dangerous Al toxicity. However, knowing that Al can and does have a significant detrimental impact on human health, even in lower quantities, it would be appropriate to continue studying the exposure of the Lebanese population to other Al sources. The obtained plasma Al concentration might not be limited to food exposure. Soil contamination by Al can affect agricultural and water sources. Food precautions and recommendations were listed for the total sample as well as for pregnant women. However, further investigation on Al medicinal and cosmetic products, food, water, and soil contamination is required.

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Author Contribution Sarine EL Daouk: conceptualization, methodology, software, writing — original draft preparation. Alain Pineau: data curation, writing — original draft preparation. M Fouad Ziade: data curation, reviewing, and editing. Raed Ezzeddine: reviewing and editing. Akram Hijazi*:* investigation, supervision. Mohamad Al Iskandarani: investigation, validation, supervision, writing — original draft preparation.

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Data Availability The data underlying this article will be shared on reasonable request to the corresponding author.

Declarations

Conflict of Interest The authors declare no competing interests.

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