Aluminum Levels in the Serum and Myometrium: A Comparison Between Women with and Without Uterine Fibroids

Farima Rahimi Mansour[1,2] iD **, Farah Farzaneh[1*]** iD **, Mohammad Mahdi Dabbagh[3], Amirreza Keyvanfar[4]**
iD

1. *Preventative Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Department of Cell and Molecular Biology, School of Biological Sciences, Kharazmi University, Tehran, Iran*
3. *Department of Physics. Sharif University of Technology, Tehran, Iran*
4. *Infectious Diseases and Tropical Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

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Corresponding Information:
Farah Farzaneh,
Preventative Gynecology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Email: farahfarzanepgrc@gmail.com

ABSTRACT

Background & Objective: Aluminum (Al) is used in different industries to produce cosmetics, supplements, drugs, food packaging, toothpaste, kitchen utensils, and antiperspirants. Uterine fibroid (UF) is women's most prevalent benign tumor during the reproductive ages. Since Al can accumulate in the body's organs, it may play a role in the pathogenesis of UF. This study aimed to measure Al levels in serum and uterine samples (normal uterine tissue of control and UF patients, and leiomyoma of UF patients).

Materials & Methods: In this descriptive study, we included ten women who underwent hysterectomy (five women due to UF and five women for a reason other than UF). Samples were obtained from serum, normal uterine tissue, and leiomyoma. Tissue and serum samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Eventually, the Al levels in samples were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Results: Al level was higher in the serum of the control group compared with UF patients (326.8 ± 360.8 µg/L vs. 211.2 ± 56.4 µg/L, P =0.310). Al level was higher in the control group compared with the normal tissue of UF patients (410.2 ± 244.7 µg/L vs. 300 ± 138.0 µg/L, P=0.465). Besides, leiomyoma had a higher Al level compared with the normal tissue of UF patients (1482.2 ± 2062.9 µg/L vs. 300 ± 138.0 µg/L, P =0.138).

Conclusion: The results showed that Al levels of tissue and serum samples in various groups differed, but these differences were not statistically significant.

Keywords: Aluminum, Myoma, Oxidative Stress, Uterine Fibroid

Introduction

Aluminum (Al), the most abundant metal in the Earth's crust, is found in minerals, rocks, and soil **(1)**. It is demonstrated that Al at a concentration of $10^{-5}M$-$10^{-4}M$ acts as a metalloestrogen, leading to estrogenic effects via alterations of gene expression and/or modifying the activity of estrogenic receptors. Metalloestrogens have a long biological half-life, can bioaccumulate, mimic the physiologic function of estrogen, and have an affinity for estrogen receptors. Additionally, Al salts upregulate estrogen receptors **(2)**. This heavy metal can reach systematic circulation through dermal absorption, ingestion, and intramuscular injection **(3)**. Al accumulates in the various organs such as the bones, brain, liver, and kidneys **(4)**. Al is used in different industries to manufacture cosmetics, supplements, Al-based antacids, medications like aspirin, intravenous solutions, dialysis fluids, and vaccine preparation as an adjuvant, antiperspirants, deodorants, food packaging,

toothpaste, and kitchen utensils **(1, 5, 6)**. According to the literature, by daily consumption of antiperspirant sprays 50-75 mg, and by taking each liter of drinking water 0.07 mg of Al enters the body. Natural sources, cooked spinach, food cooked in aluminum pots, antacids, buffered aspirin, antidiarrheal drugs, and vaccines contain 2-5 mg/day, 25 mg/kg, 6-11 mg/kg, 35-200 mg/dose, 9-50 mg/dose, 36-1450 mg/dose, and 0.15-0.85 mg/dose of Al, respectively **(7)**.

Uterine fibroid (uterine leiomyoma, UF) is the most prevalent benign gynecologic tumor, which affects women during reproductive ages. UF develops in the myometrium or muscular layer of the uterus. It comprises transformed smooth muscle cells, connective tissue fibroblasts, and many extracellular matrix (ECM) proteins, resulting in the rigid nature of UF. The symptoms of UF include pelvic pain, pressure on the bladder, sexual dysfunction, dysmenorrhea,

abnormal vaginal bleeding, and infertility. UF can be seen in both non-pregnant and pregnant women, which may lead to preterm labor, postpartum hemorrhage, and recurrent pregnancy loss. Hysterectomy and myomectomy are the definitive treatment for symptomatic fibroids **(8-12)**. Progesterone and its receptors directly stimulated UF growth, whereas estrogen and its receptor only sustained progesterone receptor expression in UF tissue **(13)**. The cause of UF has not yet been fully identified. Studies have shown that high levels of female hormones, family history, African ancestry, early age of menarche, overweight, and environmental factors can also play a role in the development and/or progression of UF **(14)**.

The number of studies that have addressed the efficacy of Al on the female reproductive system is limited. Therefore, as a pilot study, we focused on the Al levels measurement in serum and uterine samples (normal uterine tissue of control and UF patients, and leiomyoma of UF patients).

Methods

Ethical approval and study population

The ethics committee of Shahid Beheshti University of Medical Sciences approved this study (IR.SBMU.RETECH.REC.1400.869). This study was conducted at Imam Hossein Hospital from May to November 2021. Informed consent was obtained from all patients in the study group.

This descriptive study included ten women who underwent hysterectomy (five women due to UF and five women for a reason other than UF), between 25-55 years old, with no underlying disease. The exclusion criteria were: alcohol consumption, smoking habits, drug history, and multi-vitamin and mineral supplements consumption.

Sample collection

For serum samples collection, 10 ml of blood was collected from the median cubital/cephalic veins and centrifuged at 1500×g for 10 min at $4°C$. The obtained serum was stored at $-20°C$ for analysis of the Al level. The normal and UF tissue samples were obtained from the removed uterus, washed with saline, weighed, and stored at $-20°C$ for determination of the Al level.

Determination of Al levels in serum and uterine tissue

All procedures were done at the Institute for Nanoscience and Nanotechnology (Sharif University of Technology, Tehran, Iran). All samples were dried in an oven at $80°C$ for an hour. Then, 5 g of dried serum and tissue samples were digested with 15 ml nitric acid (HNO_3; Camlab, UK) (65%) and 3 mL hydrogen peroxide (H_2O_2; Camlab, UK) (30%) (Volume ratio is 5:1), and then kept at room temperature ($25°C$) for 12 hours. Then, the mixture was heated slowly at $120°C$ for 2 hours. After cooling, distilled water was added to the mix in 50 ml volume **(15)**. Finally, the levels of Al in samples were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; SPECTRO, Germany).

Statistical analysis

Data were processed in SPSS, version 18.0, IBM, USA. Data were reported as mean and standard deviation (Mean ± SD). Variables were analyzed using the independent-samples t-test and Mann-Whitney U test, and Wilcoxon test. Also, the p-value < 0.05 was considered statistically significant in this study.

Results

The mean age of subjects was 46.4 ± 7.30 and 45.8 ± 5.45 years for the control and UF groups, respectively (P=0.887).

Al levels in serum

Table 1 shows Al levels in serum samples of two groups (control and patients with UF). Our results showed Al level was higher in the control group compared with UF patients (326.8 ± 360.8 µg/L vs. 211.2 ± 56.4 µg/L, P =0.310) (Figure 1).
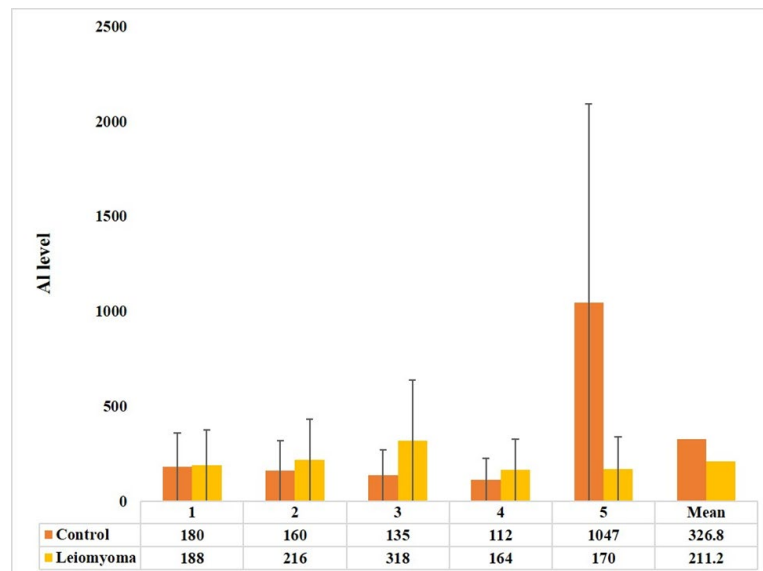
Al levels in tissue

Table 2 shows Al levels in tissue samples of two groups (control and patients with UF. Our results showed Al level was higher in the control group compared with the normal tissue of UF patients (410.2 ± 244.7 µg/L vs. 300 ± 138.0 µg/L, P =0.465). Besides, leiomyoma had a higher Al level compared with the normal tissue of UF patients (1482.2 ± 2062.9 µg/L vs. 300 ± 138.0 µg/L, P =0.138) (Figure 2).

Table 1. The levels of Al in the serum samples of the controls compared to UF patients (Mean ± SD).

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average
Al levels in the serum of controls	180	160	135	112	1047	326.8 ± 360.8 µg/L
Al levels in the serum of UF patients	188	216	318	164	170	211.2 ± 56.4 µg/L

Figure 1. The differences in Al level in serum samples (Mean ± SD, n=5) (The control group compared with UF patients: P=0.310).

Table 2. The levels of Al in the tissue samples of the controls compared to UF patients (Mean ± SD)

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average
Al levels in the normal uterine tissue of controls	287	306	230	895	333	410.2 ± 244.7 µg/L
Al levels in the normal uterine tissue of UF patients	260	362	166	537	175	300 ± 138.0 µg/L
Al levels in the myoma tissue	5580	200	190	812	632	1482.2 ± 2062.9 µg/L

	1	2	3	4	5	Mean
Control	287	306	230	895	333	410.2
Normal	260	362	166	537	175	300
Leiomyoma	5580	200	190	812	632	1482.8
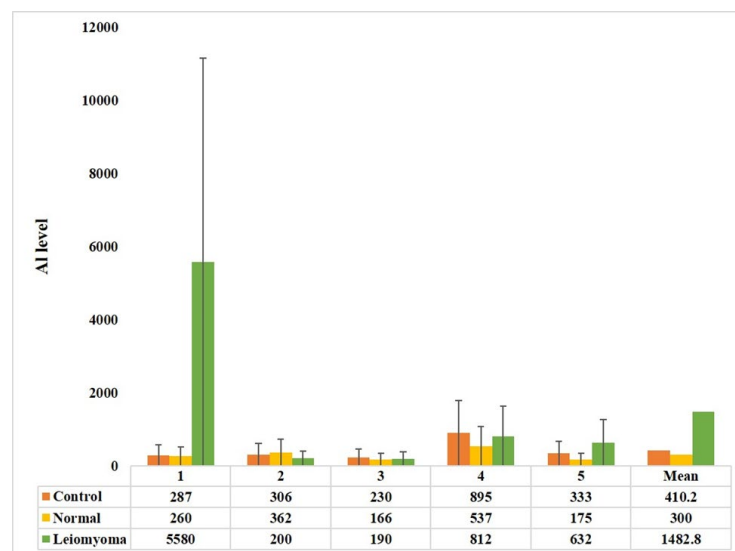
Figure 2. The differences in Al level in tissue samples (Mean ± SD, n=5) (The control group compared with the normal tissue of UF patients: p=0.465) (Leiomyoma compared with the normal tissue of UF: p= 0.138).

Discussion

Experimental evidence, both *in vitro* and *in vivo*, supports that Al causes OS and generates reactive oxygen species (ROS), which results in macromolecular damage and oxidative deterioration of lipids, proteins, and DNA (16-18). OS results from an imbalance between toxic ROS and the antioxidant system. ROS plays a role as cellular messengers in the reproductive and menopausal periods of women, which leads to the development of some conditions and diseases like age-related infertility, menopause, endometriosis, UF, and some gynecological malignancies. UF has abnormal vasculature, which results in a hypoxic microenvironment, followed by altered levels of redox enzymes. It has been shown that hypoxia and OS are critical factors in common profibrotic gynecologic disorders like fibroids, endometriosis, and postoperative adhesions. An impaired antioxidant cellular enzymatic system is the feature of UF (19, 20).

Al, as a metalloestrogen, causes gene expression changes of estrogen, progesterone, and their receptors. On the other hand, by entering to the uterine tissue and inducing OS, Al may lead to the tendency of myometrium cells to UF. However, to prove this claim, we first needed to make sure that Al was measurable in uterine tissue. So, in the primary phase, we tried to find a proper method for Al measurement in serum and uterine samples. There are different methods for measuring the Al levels in human and animal samples; some of them, are summarized below. The Al levels in the serum and the ovary of female rats were determined by graphite furnace atomic absorption spectrometry (GFAAS). The samples were digested with HNO_3 and perchloric acid ($HClO_4$) (21). Human cortex samples were digested in a microwave in a mixture of HNO_3 and H_2O_2. The Al level was measured by transversely heated graphite furnace atomic absorption spectrometry (THGFAAS) (22). HNO_3 and $HClO_4$ were used to digest male Wistar rats' liver and pancreas tissue samples. Al level was measured by spectrophotometer (23). The inductively coupled plasma-mass spectrometry (ICP-OES) was used to identify the Al levels in blood and heart tissue of male Wistar rats. Digestion was performed by hydrofluoric acid (HF) and HNO_3 (24). Thus, we did the measurement process based on the study of Sohrabi et al. (15).

We conceived that, like previous studies, Al concentration would be significantly different between control and patient groups (25-27). Nevertheless, our results show no significant difference. So, there are two ideas: 1) albeit previous studies, Al behavior is different in the uterine tissue, which normal and UF tissues are similar in Al's accumulation, and 2) the study was conducted with a small sample size, which needs more investigations. However, the present study demonstrated the procedure of Al measurement in the uterine tissue, and our hypothesis needs more research and analysis.

Limitations

Our study had some limitations. Due to the limited financial resources, this study was performed on a small sample size. This study just described Al levels in the serum and uterine tissue of participants. For determining the role of Al in the pathogenesis of UF, it is necessary to conduct an analytic study (case-control or cohort studies). So, more studies are required to explore the relationship between Al and UF.

Conclusion

We have made the first measurement of Al in the serum and uterine tissue of UF patients. The results of the study showed that Al levels of tissue and serum samples in various groups differed between normal and UF patients, but these discrepancies were not statistically significant. This may be due to the low number of participated individuals in our study. Although, further studies with bigger population are needed for a definitive statement.

Author's Contributions

F. R. M., conceptualization, study design, data collection, writing original draft; F. F., study design, supervision, critical editing; M. M. D., laboratory procedures, data collection; A. K., data analysis, critical editing.

Conflict of Interest

The authors declare no conflict of interest.

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