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EFFECT OF ALUMINIUM ON THE LEVELS OF SOME ESSENTIAL ELEMENTS IN OCCUPATIONALLY EXPOSED WORKERS*

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The mechanism of aluminium-induced cytotoxicity has not yet been defined. This study investigated possible changes in essential elements in workers occupationally exposed to Al fumes. It included 60 exposed workers and a matching control group of 60 employees not occupationally exposed to Al. Mean serum copper, calcium, zinc and iron were significantly lower in the exposed group than in controls. In addition, mean plasma and urine levels of Al were significantly higher in the exposed employees than in the controls. A statistically significant negative correlation was found between plasma and urinary Al and the studied essential elements. These findings corroborate the hypothesis that Al exposure has an adverse effect on essential elements in humans, with subsequent impact on the cellular enzymatic and metabolic processes.

KEY TERMS: *biomonitoring, copper, calcium, iron, plasma, serum, zinc, occupational exposure*

Aluminium is a serious environmental toxicant. Al toxicity generally leads to an accelerated cell death due to chronic disruption of cell metabolism. Among the effects, literature describes interference with the GTPase cycle, free radical-mediated cytotoxicity, lipid peroxidation, and changes in serum essential elements (1, 2).

Essential elements (including zinc, copper, calcium, and iron) are micro-nutrients, present in blood and tissues. They are essential for enzymatic activities and metabolic processes and even for vital functions (3, 4). Copper is essential for haemoglobin synthesis, normal bone formation, and maintenance of myelin within the nervous system. Iron is considered the most important essential elements, as its chief function is the transfer of O₂ to tissues. Calcium ion regulates neuromuscular excitability, blood coagulation, membrane integrity, and plasma membrane transport, as well as the release of hormones and neurotransmitters (3).

Zinc is intimately involved in protein, RNA and DNA synthesis (5). There is an interaction between different essential elements (5, 6). Copper deficiency is known to provoke iron deficiency and microcytic anaemia. Copper deficiency can interfere with metabolic utilisation of iron present in marginal concentrations. Interaction with toxic metals such as Al plays a major role in its toxicity. If the molecular site of an enzyme normally occupied by an essential metal is replaced by a toxic metal, toxic effects ensue. Furthermore, an overabundance of a toxic metal can interfere with metabolic utilisation of an essential metal present in normal or marginal concentrations (4, 7).

To the best of our knowledge, a limited number of studies (7, 8) have dealt with the effects of occupational exposure to Al on essential elements. The few published reports on the interaction between Al and essential metals were mostly related to patients on haemodialysis or to dietary Al intake in healthy subjects.

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The objective of our study was to assess possible changes in serum essential elements, associated with occupational exposure to aluminium, and get an idea about the degree of metabolic changes associated with this kind of exposure.

SUBJECTS AND METHODS

Subjects

This study was conducted from January to April 2005 in one of the major aluminium companies in Egypt where primary aluminium is produced from alumina by electrolysis (based on the prebake process). It included 60 exposed male workers who constituted the whole force in the production line (after application of exclusion criteria) and who worked eight hours a day. Not all workers used provided protective equipment at work. The study also included a control group of 60 male employees with no history of occupational exposure to Al, matched for age, social class and smoking habits.

Informed consent was obtained from all study subjects, and the study was approved by the appropriate bioethics committee. Subjects with liver or kidney diseases, history of bilharziasis, history of use of calcium or iron preparations or AL containing antacids, laxatives or antiperspirants were excluded from the study (4, 7). Workers were examined during the shift.

Industrial processes

Primary aluminium is produced by the electrolysis of alumina (Al_2O_3) dissolved in molten cryolite ($\text{AlF}_3 \cdot 3\text{NaF}$). The electrolysis is carried out in a carbon-lined steel shell, called *the pot*, and the building is the pot-room whose floor makes the cathode. The anode consists of carbon blocks suspended in the molten ore which are consumed and should be renewed periodically. In the prebake process, anodes are prefabricated in a nearby carbon electrode section of the same factory, and are replaced as required. Molten aluminium is tapped and transferred to the cast house for the production of ingots and cylinders. In the pot-rooms, in addition to large quantities of Al fumes and particulates of alumina, workers are also exposed to pollutants generated by the molten flux, carbon anodes and alumina feed such as hydrogen fluoride, carbon oxides, and sulphur dioxide (9).

Methods

Prior to full clinical examination, each subject of either group was interviewed using a special questionnaire on detailed medical and occupational history, as well as on lifestyle which might affect the body load of Al or essential metal levels, such as diet, alcohol consumption and smoking (7, 8).

Blood samples were obtained to determine serum copper (Cu) using the colorimetric assay (10), serum calcium (Ca) using a spectrophotometer (11) with Ca- free columns, serum zinc (Zn) using spectrophotometry without deproteinisation (12), serum iron (Fe) using colorimetry (13), as well as plasma Al concentration (14).

To determine urinary Al, urine samples were collected in acid-washed polystyrene bottles and precaution was taken to avoid contamination during collection. Both urine and blood samples were collected in the afternoon after the 8-hour work shift and before meal (dinner). Both plasma and urinary Al concentrations were determined using graphite furnace atomic absorption spectrometry. Standard addition technique was used for calibration (14).

Environmental monitoring

To determine the level of air contaminants, samples of dust were collected from the breathing zone of the workers, including different shifts and locations. Sampling and analysis were done according to the NIOSH manual of analytical methods (1979) (15). Industrial hygiene data sheet for personal sampling was used (16).

The results were collected and analysed according to standardised statistical methods. Student's *t*-test and ANOVA were used to compare quantitative data and chi-square for the qualitative data. Differences were considered statistically significant when the *P* value was <0.05 and highly significant when it was <0.01 .

RESULTS

Table 1 shows no statistically significant differences between the exposed and control groups in relation to age and smoking ($P > 0.05$). The mean age of the exposed workers was (40.38 ± 8.97) years and that of controls (39.09 ± 13.39) years. Aluminium exposure duration ranged between eight and 30 years (mean 18.08 ± 6.63). Biomonitoring clearly showed that

mean levels of Al in both plasma and urine were much higher in the exposed group [(45.9±19.77) μg L⁻¹ and (68.89±21.11) μg L⁻¹, respectively] than in controls [(3.08±3.12) μg L⁻¹ and (4.95±4.81) μg L⁻¹, respectively], and the differences were statistically significant (P>0.01).

Table 2 shows that mean serum Cu, Zn, Fe and Ca were significantly lower in the exposed workers than in controls (P>0.01).

Table 3 shows the influence of Al exposure duration on mean plasma and urinary Al, as well as on the measured essential elements (serum Cu, Zn, Fe and Ca). It is quite apparent that the longer the duration of exposure, the higher plasma and urinary Al and the lower essential elements. Again, the differences were statistically significant.

Table 4 shows that all studied essential elements significantly negatively correlate with biological exposure indices (plasma and urinary Al levels).

As regards the results of environmental monitoring, Table 5 shows that the mean levels of different environmental contaminants were within permissible levels, save for aluminium fumes which exceeded the threshold limit value (TLV) (17).

DISCUSSION

It has been proved that Al is involved in the pathogenesis of many clinical disorders in humans, such as neurobehavioral disorders, osteomalacia and microcytic anemia. Aluminium-induced cytotoxicity

Table 1 Characteristics of the study population

Variable	Exposed group (n = 60)	Control group (n = 60)	Test of sig.	P-value
Age / years; mean±S.D	40.38±8.97	39.09±13.39	t-test = 0.63	>0.05
Duration of exposure to Al / years; mean±S.D	18.08±6.63	--	--	--
Smoking habits				
Smokers / No., (%)	28 (46.7)	25 (41.7)	X ² = 0.5	>0.05
Ex-smokers / No., (%)	6 (10)	7 (11.7)	X ² = 0.4	>0.05
Non-smokers / No., (%)	26 (43.3)	28 (46.6)	X ² = 0.11	>0.05
Plasma Al / μg L ⁻¹ ; mean±SD	45.9±19.77	3.08±3.12	t-test = 16.59	3.08±3.12
Urine Al / μg L ⁻¹ ; mean±SD	68.89±21.11	8.99±4.81	t-test = 7.67	<0.01

Table 2 Serum levels of Cu, Zn, Fe and Ca in the studied groups

Variable	Exposed (n = 60) mean±SD	Control (n = 60) mean±SD	t-test	P value
Serum Cu / μg dL ⁻¹	82.09±9.18	95.06±13.12	6.27	<0.01
Serum Zn / μg dL ⁻¹	77.98±13.88	86.33±9.13	3.89	<0.01
Serum Fe / μg dL ⁻¹	83.13±24.97	99.44±17.99	4.11	<0.01
Serum Ca / mg dL ⁻¹	8.55±1.33	11.01±1.55	9.33	<0.01

Table 3 Mean levels of the studied parameters in the exposed group according to their duration of exposure

Variable	Duration of exposure / year			ANOVA test	P value
	<10 (n = 13) mean±SD	10 to 20 (n = 23) mean±SD	>20 (n = 24) mean±SD		
Plasma Al / μg L ⁻¹	9.8±12.44	28.01±21.07	45.81±28.12	15.93	<0.01
Urine Al / μg L ⁻¹	18.88±18.48	45.55±33.05	66.8±35.71	12.89	<0.01
Serum Cu / μg dL ⁻¹	92.85±11.54	86.95±11.22	81.99±14.92	3.21	<0.05
Serum Zn / μg dL ⁻¹	87.33±14.04	82.73±10.26	76.03±9.56	6.12	<0.01
Serum Fe / μg dL ⁻¹	100.88±24.05	85.09±16.66	82.66±25.04	3.46	<0.05
Serum Ca / mg dL ⁻¹	9.98±1.33	9.25±1.66	8.50±1.84	8.98	<0.01

Table 4 Correlation coefficient between biological aluminum exposure indices and serum essential metals

Variable	Serum Cu			Serum Zn			Serum Fe			Serum Ca		
	r	r ²	F	r	r ²	F	r	r ²	F	r	r ²	F
Plasma Al	-6.66*	0.43	57.55	-0.54*	0.29	32.44	-0.61*	0.37	48.55	-0.88*	0.78	274.86
Urine Al	-0.63*	0.39	49.48	-0.54*	0.30	35.71	-0.60*	0.36	46.91	-0.88*	0.77	267.87

* Significant correlation $P < 0.05$

Table 5 Concentrations of environmental pollutants in the work place

Air contaminants	Mean ± SD	TLV
Fluoride particulate / mg m ⁻³	1.99 ± 0.3	2.5
Hydrogen fluoride / ppm	2.01 ± 0.5	3
CO / ppm	41.8 ± 5.0	50
SO ₂ / ppm	1.8 ± 0.2	2
TSP / mg m ⁻³	8.5 ± 2.9	10
Aluminum fumes / mg m ⁻³	6.91 ± 1.8*	5

TSP: Total suspended particulates (including AL₂O₃)

* More than TLV

TLV = Threshold limit value

due to chronic disruption of cell metabolism was recently described as the cause of these clinical disorders (18, 19).

In terms of biomonitoring, it is not yet clear which of the two measurements of Al concentration is more suitable for medical evaluation of Al exposure, urine or plasma. However, some authors suggest to use both matrices (20, 21) for persons with high or chronic occupational exposure. In our study, both parameters were monitored, especially as we established that the concentration of Al fumes in the working environment were higher than the TLV. Not only were both parameters significantly higher in the exposed group than in controls, but they were also much higher than the upper normal limit of Al in plasma and urine ($\geq 10 \mu\text{g L}^{-1}$ in plasma and $\geq 25 \mu\text{g L}^{-1}$ in urine (Table 1).

We found that occupational Al exposure was associated with changes in the serum concentrations of Cu, Zn, Fe and Ca in the exposed group. All elements were significantly lower than in controls. Our results are in accordance with those reported by others who confirmed that serum Al concentration influenced the metabolism, serum concentrations and distribution of essential metals in the body tissues (7). They also found a significant decrease in serum Fe, Cu and Zn in Al-exposed employees in comparison with controls, even though Al concentrations in ambient air were below the threshold limit, and the increase in plasma Al was only slightly higher than in controls.

There is no satisfactory explanation for the reduction of serum concentrations of essential elements associated with chronic Al exposure. It was speculated that high plasma Al which binds essential elements, especially copper and zinc, and which also induces metallothionein which binds many heavy metals (22, 23). Others however (4, 7) reported that Al-induced hypozincaemia might be due to the redistribution rather than total body decrease, as it may be accompanied by normal Zn concentrations in the hair, testes, heart and liver.

As most toxic and essential metals share chemical properties, it has been suggested that a number of metabolic interactions takes place between essential and toxic metals (like Al), which could reduce the levels of essential metals or increase the health risks associated with toxic metals (4).

Alumina exposure in primary Al industry was reported to have a specific effect on serum iron (23). Not only was aluminium exposure found to decrease serum iron and the percentage of transferrin saturation, but also to interfere with iron incorporation in the hem group, increasing free erythrocytic protoporphyrin and resulting in microcytic hypochromic anaemia (24). A competition between both metals takes place due to the fact that both Al and Fe share certain features in their biochemistry, the common route of absorption, plasma transport protein (transferrin), and metabolism (19, 24). It should be mentioned that Al neurotoxicity might be related in part to the disruption of the

normal Fe homeostasis and Fe-dependent cellular metabolism in the brain (25).

As regards hypocalcaemia associated with occupational Al exposure, the same was observed by other workers (26, 27) who reported that Al exposure greatly increased the risk of fragility fractures and bone disorders due to the fact that Al decreases serum Ca and inhibits bone mineralisation. They also suggested that Al might interfere with Ca uptake by enterocytes through a general effect on cell membrane, leading to a reduced transcellular Ca absorption in the small intestine.

Experimentally, high levels of alkaline phosphatase were found due to increased osteoplastic activity, provoked by the disturbance of bone formation caused by long-term exposure to Al (28). It was suggested that the toxic effects of Al could be mediated through modifications in the intracellular Ca homeostasis, and result in altered neuronal and other cellular functions (26).

Occupational history and the duration of exposure to Al could be considered the prime measure for Al exposure (20, 29). The longer the duration of exposure to Al, the higher the urinary and plasma Al levels that could be monitored among the exposed workers. Also, workers with long-term exposure to Al had lower serum levels of essential elements than did workers after brief exposure (7), which provides a further support to our findings and clarifies the importance of the effect of duration of exposure to Al on workers' health.

In conclusion, this study clearly shows that occupational Al exposure has an adverse effect on the levels of serum essential elements with subsequent impact on the cellular enzymatic and metabolic processes. However, the toxic mechanism by which Al acts still needs further investigation. We therefore believe that multidisciplinary collaborative research efforts should be encouraged, involving scientists from different specialties. Emphasis must be placed on increasing our understanding of the chemistry of Al in biological systems, and on understanding the cellular and molecular mechanisms of Al toxicity.

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Sažetak

INTERAKCIJA ALUMINIJA S ESENCIJALNIM ELEMENTIMA U PROFESIONALNO IZLOŽENIH RADNIKA

Dosada nije razjašnjen mehanizam citotoksičnoga djelovanja aluminija. U ovome ispitivanju pokušalo se ustanoviti dolazi li do promjena u sadržaju esencijalnih elemenata u skupini radnika koji su bili profesionalno izloženi aluminijskim parama. Ispitivanje je obuhvatilo 60 izloženih radnika i odgovarajuću kontrolnu skupinu od 60 zaposlenika koji nisu bili profesionalno izloženi aluminijskim parama. Rezultati su pokazali značajno niže srednje razine bakra, kalcija, cinka i željeza u serumu izloženih radnika u odnosu na kontrolu. Usto su iskazali značajno više razine Al u plazmi i mokraći. Utvrđena je statistički značajna negativna korelacija između Al u plazmi/mokraći i ispitanih esencijalnih elemenata. Ovi rezultati upućuju na pretpostavku da izloženost aluminiju nepovoljno djeluje na razine esencijalnih elemenata u ljudi, a time i na lučenje enzima i stanični metabolizam.

KLJUČNE RIJEČI: *biomonitoring, bakar, cink, citotoksičnost, kalcij, plazma, profesionalna izloženost, serum, željezo*

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