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Occupational exposure to aluminum and its amyloidogenic link with cognitive functions

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ABSTRACT

As many other metals, aluminum is a widely recognized neurotoxicant and its link with neurodegenerative disorders has been the subject of scientific debate. One proposal focuses on amyloid β deposition (amyloidogenesis) as the key player in triggering neuronal dysfunction the so-called amyloid cascade hypothesis. We undertook this study first to investigate the cognition status of workers exposed to Al dust in an Al factory in Southern Cairo, second, to evaluate serum amyloid precursor protein (APP) and cathepsin D (CD) enzyme activity to study the possible role of Al in amyloidogenesis, and finally to explore the relation between these potential biomarkers and cognitive functions. The study was conducted on 54 exposed workers and 51 matched controls. They were subjected to questionnaire, neurological examination and a cognitive test battery, Addenbrooke's Cognitive Examination — Revised (ACE-R). Serum Al, APP and CD enzyme activity were measured. A significant increase of serum Al was found in the exposed workers with an associated increase in serum APP and decrement in CD activity. The exposed workers displayed poor performance on the ACE-R test. No significant correlation was detected between ACE-R test total score and either APP or CD activity. We concluded that occupational exposure to Al is associated with cognitive impairment. The effect of occupational Al exposure on the serum levels of APP and CD activity may be regarded as a possible mechanism of Al in amyloidogenesis. However, our findings do not support the utility of serum APP and CD activity as screening markers for early or preclinical cognitive impairment.

1. Introduction

Aluminum (Al) is a ubiquitous element in nature. It is the third most common element in the earth's crust and causes unavoidable environmental exposure. Since the metal has no essential function in the mammalian organism but is toxic under special circumstances, it can be regarded as a harmful contaminant for humans. However based upon its physical and chemical properties Al has gained wide industrial and commercial importance [1]. Occupational exposure to Al occurs during refining of the primary metal and in secondary industries that use Al products. Secondary Al smelters involve recycling Al products and scrap [2]. Depending on the process and impurities contained in scrap Al, ambient air in Al smelters may contain a multitude of compounds in addition to Al [3].

Al is a well-established neurotoxicant and is suspected to be linked with various neurodegenerative diseases including Alzheimer's disease (AD) [1]. The etiological factors of AD are not clearly known, although several hypotheses had been studied and some were proved including genetics, head trauma, oxidative stress, infectious agents, and environmental factors [4].

Alzheimer's disease is a progressive neurodegenerative disorder and the most common form of dementia. The pathological hallmarks of AD are the deposition of extracellular senile plaques, intracellular neurofibrillary tangles (NFTs), and the selective loss of synapses and neurons in the hippocampal and cerebral cortical regions [5]. The major component of NFTs is the phosphorylated tau protein, while senile plaques are largely comprised of amyloid beta peptide (AβP). AβP is generated via sequential proteolytic cleavage of amyloid precursor protein (APP), mainly through β-secretase and γ-secretase enzymes [6].

The scientific literature contains numerous studies that discuss the link between Al and various neurodegenerative disorders [7–9]. The possible relation between Al and AD was hypothesized based on epidemiological studies relating the Al content of drinking water with increasing incidence of dementia and AD [10,11]. Further, Al was suggested to be the cause of dialysis-associated encephalopathy (DAE) [12]. However, the DAE pathology was found to be clearly different from AD pathology through histological analyses of dialysis patients by light microscopy [13]. Worldwide, the last outbreak of Al toxicosis was reported in 2001 in Curaçao where 10 patients died. The incident

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was associated with a cement mortar distribution pipe from which Al and calcium leached into the water used to prepare the dialysate [14]. In Canada, Neri and Hewitt reported that Al concentration over 0.2 mg/L in water may increase the incidence rate of AD (odds ratio = 1.46) [15]. However, fluorine content, rather than Al, was found to be more correlated with AD [16]. Similarly no significant relationship was obtained for Al effect in drinking water in several other studies [17,18].

Regarding occupational Al exposure, studies did not produce consistent results regarding cognitive impairment. Polizzi et al. found a negative relationship between serum Al levels and mini-mental state exam (MMSE) and the clock drawing test scores; there was a positive relationship between serum Al levels and both test times [19]. Contradictory findings were deduced by other studies which have raised the question as to whether the metal may play a role in these neurological disorders [20–22].

Aβ peptide and APP accumulation in the brain are the key factors in initiation and progression of amyloidogenesis in neurodegenerative diseases. APP gene was one of the seven genes found to be significantly up-regulated by Al ions in human neural cells [23]. The best model for the diagnosis of incipient AD was cerebrospinal fluid (CSF) sAPP and tau, with a sensitivity of 95.20% and a specificity of 81.20%. Indeed the diagnostic utility of these markers was supported [24]. However several other studies did not support such conclusion [25,26]. Research data suggest that Al may modulate the expression and processing of APP [27,28]. Indeed chronic oral ingestion of Al gradually accumulated in brain regions and was sufficient to increase APP levels and launch the cascade that resulted in the formation of amyloid plaques in the brain [29].

Peripheral changes of APP cleavage products may be more closely related to cerebral changes due to AD than to a pathological response in the periphery [30]. Increased concentrations of full-length APP protein in blood platelets and APP mRNA levels in blood mononuclear cells have been reported in AD [31,32]. Moreover plasma APP levels mirrored the changes observed with CSF levels in AD [33,34].

Cathepsin D (CD) is an active acid protease that is initially produced as a nonfunctional enzyme in the trans Golgi network in the rough endoplasmic reticulum and undergoes various proteolytic transformations until it reaches its targeted intracellular vesicular structure where it is involved in intracellular protein breakdown. The functions of CD are to hydrolyze APP protein and to clear Aβ peptide from the central nervous system. As such, CD might involve in the pathogenesis of AD [35,36]. Indeed variants of CD gene can impede the functions of proteolytic degradation, thus increasing the risk of AD. This gene polymorphism was significantly associated with the general intelligence of healthy elderly [37], with the T allele (CD-C/T gene) considered as a high-risk factor for developing AD [38,39]. However these results were not replicated in many other studies [40–43].

One of the mechanisms by which Al causes its neurotoxicant effect is through inhibition of protein functions and enzymatic activities. Studies using neuroblastoma cells or rat cortical neurons have described endocytosis of AL with its accumulation inside lysosomes [44,45]. Further, Aβ peptides were accumulated and degraded in the lysosomes of the microglia by CD in lysosomes. Nakanishi described the pathological roles of neuronal and microglial cathepsins in brain aging and age related diseases [46]. The potential of Al to interact and disrupt Aβ peptide catabolism via the inhibition of its proteolytic degradation by CD was demonstrated [47].

Currently neurodegenerative disorders are diagnosed by a multitasking process involving neuropsychological tests, imaging and CSF assessment. CSF biomarkers include total tau protein (t-tau), phosphorylated tau (p-tau), and the 42 amino acid isoform of Aβ (Aβ42) [48]. However, CSF biomarkers, though clinically validated, are not routinely used and are not ideal in a perspective of mass-screening in the general or at risk population. To this respect, blood-derived circulating biomarkers would be a better solution. Possibly because at peripheral level, the clinical picture is variable, blood biomarkers have not yielded consistent, easily reproducible or sensitive levels for diagnosis, evaluation of disease progression, or treatment effects [48,49]. However promising results were presented by Ray et al. who developed from a panel of 18 plasma circulating molecules an algorithm for discriminating AD patients from controls and progression of mild cognitive impairment (MCI) subjects to AD [50].

Accordingly we undertook this study first to investigate the cognition status among workers occupationally exposed to Al dust in an aluminum smelter and production lines, second, to evaluate the serum APP and CD enzyme activity in exposed workers as a possible mechanism of Al role in amyloidogenesis, and finally to explore the relation between these potential biomarkers and cognitive functions and their possible utility as screening tools for early cognitive impairment in Al exposed workers.

2. Materials and methods

2.1. Materials

This cross sectional study was conducted on the whole production working population (n = 54) in one of the major aluminum factories in Helwan area, Southern Cairo. The factory is producing anodized and electro-static powder coated aluminum profiles for tubes, transportation systems including busses and trucks and many other applications. The factory constitutes many departments namely, extrusion presses, anodizing, electrostatic powder coating and Al smelters (primary and secondary).

All eligible employees were invited to participate in the study. Eligibility criteria for exposed workers included regular employment in the factory for at least the preceding 5 years. Those who met the criteria for inclusion were the exposed population and they comprised 54 workers (23 from the Al smelters and 31 from other production lines in the factory). Fifty one controls were recruited from workers in simple low rank administrative jobs that did not carry the risk of exposure to Al (porters, clerks, security personnel and switch operators). The controls were chosen so as to be matched with exposed workers regarding age, sex, educational level and smoking status.

Exclusion criteria for both the exposed and control workers were: any history of alcohol intake or drug abuse and regular intake of medications for hyperacidity as they have high Al content, and any condition that may cause cognitive impairment including liver, kidney, cerebrovascular diseases and uncontrolled diabetes. Workers with marked poor vision were excluded to avoid poor performance in the Addenbrooke's Cognitive Examination.

2.1.1. Ethical considerations

All participants were literate. Subjects were treated according to the Helsinki Declaration of biomedical ethics. Informed consent was obtained from all subjects after proper orientation regarding the objectives of the study, data confidentiality and the impact of the study. The study was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University (N-54-2012).

2.2. Methods

2.2.1. A specially designed questionnaire

A specially designed questionnaire was administered during an in-person interview. We investigated confounding variables considered to be possible risk factors for cognitive impairment. The questionnaire consisted of detailed questions regarding self-reported illness, health and well-being, and life-style habits such as smoking, alcohol consumption, medications, occupation and work environment. Medical history included major psychiatric illnesses, chronic neurological diseases such as cerebrovascular stroke, Parkinsonism, epilepsy, intracranial neoplasms, major medical diseases such as renal, hepatic, metabolic

disorders, endocrinal disorders, and collagen vascular diseases. Participants were subjected to both general and neurological examinations.

2.2.2. Addenbrooke's Cognitive Examination — Revised (ACE-R)

The ACE-R is a brief cognitive test battery that assesses five cognitive domains, namely attention/orientation, memory, verbal fluency, language and visuospatial abilities. Total score is 100; higher scores indicate better cognitive functions. The test version was translated into Arabic and back translated with reliability calculated by Cronbach's alpha test to be 0.822 in exposed workers and 0.792 in the controls. Administration of a single ACE-R test needs, on average, 45 min. The test was administered by a specially trained neurologist who was not aware of the levels of serum Al, and who assisted the participant in the questionnaire sheet.

2.2.3. Biochemical investigations

2.2.3.1. Sample collection. Specimens were collected in an area away from the work environment. Venipuncture was performed, and the blood was collected into plain tubes certified free of the trace element. Samples were allowed to clot for 2 h at room temperature and they were centrifuged for 20 min at 1000 × *g*. The serum was poured into a plastic trace element shipping container and was stored at −80 °C until assay.

Serum concentration of aluminum was determined by electrothermal atomic absorption spectrometry [51]. The results were expressed in μg/L.

Serum concentration of APP was measured using APP Human Elisa Assay (Boster Biological Technology, Ltd. USA), according to the manufacturer's instructions [52]. The results were expressed in ng/mL.

Cathepsin D activity was assayed in serum using CD activity kit (BioVision, Mountain View, CA USA). Briefly, 50 μL of human serum was added to each well of a 96-well plate. A master mix comprised of a 1:25 dilution of CD substrate: reaction buffer was made (not antibody based), and 52 μL of this mix was added to each assay well. The plate was incubated for 1 h at 37 °C and read with a fluorometer (Fluoroskan Ascent FL2.6) using excitation and emission filters of 328 and 460 nm, respectively. Values were properly gain adjusted, and reported as relative fluorescence units (RFUs) [53].

2.2.4. Workplace monitoring

Air sample estimates of total dust level, Al and other metal fractions were assessed by the industrial hygienist of the factory. The authors conducted site visits to rule out confounding exposures such as solvents, and evaluated the job tasks and occupational exposures of the workers. The work place monitoring was performed according to the OSHA standards and procedures [54].

2.2.5. Statistical analysis

The data were coded and entered using the statistical package SPSS 15.0 for windows (SPSS Inc., Chicago, IL, USA, 2006). Data from groups were compared using two tailed Student's *t* test and Chi square test as appropriate. Analysis of variance (ANOVA) and post-hoc test (Bonferroni) were used for multiple comparisons between the groups. The Pearson correlation test was used to test the correlation between different variables among the exposed groups. Multivariate analysis of variance (MANOVA) was done to determine if the response variables (cognitive functions) are altered by the independent variable (exposure to aluminum). Multivariate analysis of covariance (MANCOVA) was done when age and educational level were added as covariates, to show their effects on cognitive functions. The statistical significance was defined as P value < 0.05.

3. Results

Workplace monitoring showed that the mean level of total dust was 24.8 ± 3.5 mg/m^3 in smelter department and 16.5 ± 1.5 mg/m^3 in the other departments. Total Al dust fraction constituted about 38% (this proportion varied between 15 and 43%, with a standard deviation of 5%) with mean level of 10.3 ± 2.4 mg/m^3 in the smelter department and 6.5 ± 1.5 mg/m^3 in the other departments (mean for 8 h time weighted average). Metals other than Al collectively constituted about 31% of the total dust (lead, copper, zinc, manganese, iron, chromium), individual metal levels were far less than the permissible exposure levels set by the Egyptian Environmental Protection Agency (EPA).

Regarding clinical manifestation among the study population (data are not presented), none of the study groups showed manifestations suggestive of neurological or cognitive disorders. Blood lead levels were measured to exclude one possible confounder and none of the workers exceeded the permissible blood lead level of 40 μg/dL (range: 10.3 μg/dL–21.4 μg/dL, mean (SD): 16.30 ± 4.2).

Table 1 summarizes demographic characteristics in studied groups. Both exposed ($n = 54$) and non-exposed control groups ($n = 51$) were matched as regards age, smoking and years of education. Regarding clinical manifestation among the study population (data are not presented), none of the study groups showed manifestations suggestive of neurological or cognitive disorders.

Fig. 1 shows comparisons of ACE-R test score, serum Al, APP and cathepsin D activity between these 2 groups, there were statistically significant lower levels of total ACE-R test score and serum cathepsin D activity in the Al exposed workers versus non-exposed control (82.65 ± 6.91 and 15.99 ± 6.80 versus 93.73 ± 3.32 and 24.3 ± 5.01 respectively, $P < 0.001$) and statistically significant higher levels of serum Al and serum APP in the exposed workers than in the non-exposed control (20.27 ± 9.62 and 97.98 ± 17.04 versus 4.43 ± 2.07 and 40.69 ± 8.79 respectively; $P < 0.001$).

To investigate the relation between the degree of occupational exposure to Al based on the Al total dust level and the internal Al dose (serum level), we further subdivided the exposed workers into a high exposure group ($n = 23$) in the smelter department (Al total dust level was 10.3 ± 2.4 mg/m^3) and intermediate exposure group ($n = 31$) that comprised workers in other departments (Al total dust level was 6.5 ± 1.5 mg/m^3). ACE-R test score, serum Al, APP and cathepsin D activity were compared between the two exposed subgroups (Table 2). Serum Al and APP were significantly higher in the smelter group (26.52 ± 8.94 and 105.87 ± 16.20 respectively) than in the other subgroup (15.64 ± 4.29 and 92.13 ± 15.42 respectively, $P < 0.001$). There was no statistically significant difference between smelter group and other departments as regards total ACE-R test and serum cathepsin D activity.

Table 3 depicts ACE-R test total score and subtest scores namely orientation, memory, language, verbal fluency, and visuospatial & perceptual abilities among exposed and control groups. Statistically significant lower scores were recorded in the exposed workers, denoting cognitive impairment. Apart from attention/orientation domain, all domains were affected.

Table 1
Demographic characteristics in studied groups.

	Exposed N = 54	Control N = 51	P
Age (years)	45.63 ± 10.08	46.31 ± 12.06	NS
Range	23–58	20–57	
Smokers no. (%)	15(27.8%)	16(31.4%)	NS
Education (years)	13.33 ± 2.57	14.02 ± 2.77	NS
Duration of work (years)	21.65 ± 11.03	–	

Data were presented as mean ± SD.
NS: Non-significant.

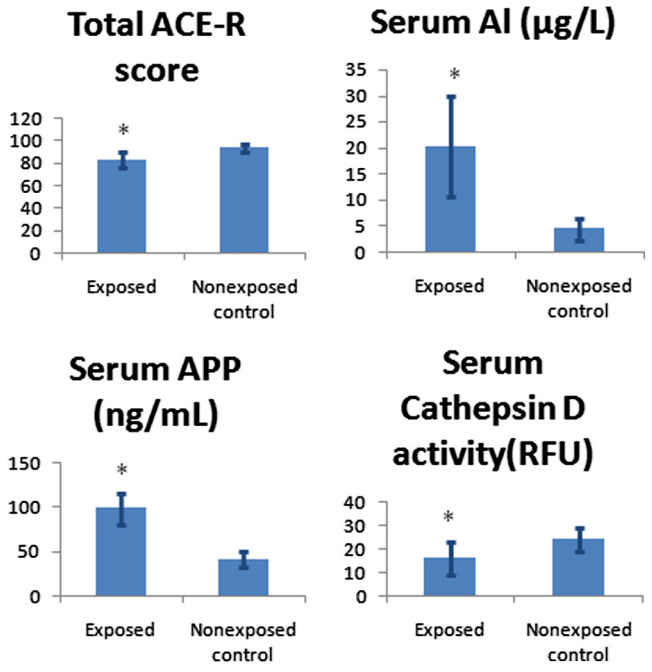

Fig. 1. Comparison of ACE-R test score, serum Al, APP and cathepsin D activity in studied groups. Results are presented as mean ± SD. *Values differ significantly from non-exposed control ($P < 0.001$).

The median level of ACE-R total score among exposed workers was 83.5. This score was used as a cutoff level to subdivide the exposed workers into a group of higher ACE-R score ≥83.5 (n = 25, 46.2%) and a group of lower score <83.5 (n = 29, 53.7%). Table 4 shows that serum Al level was significantly higher in the lower score ACE-R group compared with the group of higher score (22.86 ± 11.10 and 17.28 ± 6.59 respectively, $P < 0.05$). Regarding APP and cathepsin D activity, there was no statistically significant difference between the 2 exposed subgroups.

Correlations between different variables (age, duration of work and total ACE-R test score with serum Al, APP and cathepsin D activity) in the exposed group were shown in Table 5. There was a significantly positive correlation between APP and both duration of work and serum Al ($P < 0.01$). On the other hand, a significant negative correlation was found between serum cathepsin D activity and both serum Al and APP ($r = -0.274, -0.558$ & $P < 0.05, <0.001$, respectively). Another significant negative correlation was detected between total score of ACE-R test and both duration of work ($r = -0.391$, $P = 0.003$) and serum Al ($r = -0.317$, $P = 0.02$).

Multivariate regression analysis was done to test for significant independent predictors for cognitive impairment (ACE-R test). Al was found a significant predictor ($P = 0.02$, coefficient $\beta = -2.27$), while APP and cathepsin D were non-significant predictors for cognitive impairment ($P = 0.55, 0.87$ and coefficient $\beta = -0.033, -0.022$ respectively).

Table 2
Comparison of ACE-R test score, serum Al, APP and cathepsin D activity in subgroups of exposed workers.

	Smelter workers (N = 23)	Other departments' workers (N = 31)
Total ACE-R score	80.83 ± 6.69	84.0 ± 6.87
Serum Al (μg/L)	26.52 ± 11.14	15.64 ± 4.29(a)***
Serum APP (ng/mL)	105.87 ± 16.20	92.13 ± 15.42(a)***
Serum cathepsin D activity (RFU)	14.21 ± 7.21	17.30 ± 6.27

Results are presented as mean ± SD.
(a) Values differ significantly from Smelter workers.
ACE-R = Addenbrooke's Cognitive Examination – Revised; APP = amyloid precursor protein, RFU = relative fluorescence unit.
*** Denotes significance ($P < 0.001$).

To explore the confounding effect of certain covariates (age and educational level) on cognitive functions in the studied groups, multivariate analysis of variance (MANOVA) was applied prior to covariate inclusion. A highly significant cognitive impairment was reported in exposed workers compared to their referent controls ($\lambda = 0.4$, $F(1,103) = 36.8$, $P < 0.001$). These were confirmed across cognitive parameter scores tested ($P < 0.001$, $F(1,103) = 106.51, 51.992, 30.14$ and 107.33), for total memory, total language, total visual spatial &perceptual ability, and total ACE-R test scores respectively. The power to detect the effect was 1.000. When age and educational level were added as covariates in a multivariate analysis of covariates (MANCOVA), these effects became non-significant ($\lambda = 0.948$, $F(4,96) = 1.305$, $P = 0.274$). The effect of smoking on cognitive impairment was also tested using MANOVA. It showed that there was no significant effect on the different cognitive parameters of the ACE-R test [$\lambda = 0.975$, $F(4,100) = 0.628$, $P = 0.644$]. These results were applied to all parameters of the ACE-R test.

4. Discussion

Aluminum is the most widely distributed metal in the environment and is extensively used in modern daily life. This ubiquitous exposure made the total body burden of Al in healthy human subjects approximately 30–50 mg where, the brain is an important accumulation site for Al whatever the route of exposure with age related increase in brain Al level [55,56]. In this study serum Al was estimated as an index for long term exposure. The significantly increased levels of serum Al in exposed workers versus their referent controls go in accordance with the results obtained elsewhere who considered Al levels in non-exposed individuals to be <25 μg/L in urine and <10 μg/L in plasma [57,58].

To investigate the relation between the degree of occupational exposure to Al based on the Al total dust level and the internal Al dose (serum level), we further subdivided the exposed workers into a high exposure group in the smelter department (Al total dust level was 10.3 ± 2.4 mg/m^3) and intermediate exposure group that comprised workers in other departments (Al total dust level was 6.5 ± 1.5 mg/m^3). Higher degree of exposure was reflected on the serum level of Al. Cumulative exposures to Al are not easy to estimate. Indeed exposure levels cannot be correlated to serum or urinary levels very accurately and in many cases exposure started long before the toxicokinetics of Al were considered to be of any significance [59].

Occupational exposures in Al smelters include in addition to Al other metals: Zn, Pb, Cu, and Fe [2]. The role of metal ions as a driving force to modulate the precipitation of Aβ was investigated. Mounting evidence is demonstrating roles for the APP and its proteolytic product Aβ in metal homeostasis. Aberrant metal homeostasis is observed in patients with AD, and this may contribute to AD pathogenesis, by enhancing the formation of reactive oxygen species and toxic Aβ oligomers and facilitating the formation of the hallmark amyloid deposits in the brain. On the other hand, abnormal metabolism of APP and Aβ may impair brain metal homeostasis as part of the AD pathogenic process [60]. Singh et al. demonstrated that Cu's effect on brain Aβ homeostasis depends on whether it is accumulated in the capillaries or in the parenchyma. Indeed, the corrupted metabolism of Aβ in AD may cause severe perturbances of essential metal homeostasis [61]. In transgenic mouse brain age-related increases in copper, iron, and cobalt levels contributed to the age-dependent formation of amyloid peptide and oxidative damage. Moreover, APP and Aβ expression modulated metal levels, particularly copper [62].

Adverse neurological outcomes as a result of occupational Al exposure can be estimated in a number of different ways including; exposure grading for different job categories, number of years working in the Al industry, and ever versus never worked in the Al industry. In this study all these approaches were applied to evaluate the effect of Al on cognitive functions. The level of Al in the exposed workers was

Table 3
ACE-R test total score and subtest scores namely orientation, memory, language, verbal fluency, and visuospatial & perceptual abilities among Al exposed workers and non-exposed control group.

Domain	Max score	Exposed N = 54	Control N = 51	P
Total orientation/attention	18	18	18	–
Memory	26	20.67 ± 2.80	25.08 ± 1.23	<0.001
Immediate recall	3	2.15 ± 0.81	2.9 ± 0.30	<0.001
Antegrade	7	6.43 ± 0.98	6.98 ± 0.14	<0.001
Retrograde	4	4	4	–
Delayed recall	7	3.43 ± 1.929	6.22 ± 1.22	<0.001
Memory recognition	5	4.67 ± 0.67	4.98 ± 0.14	0.001
Language	26	24.39 ± 1.37	25.86 ± 0.49	<0.001
Reading sentences	1	1.0 ± 0.00	0.98 ± 0.14	n.s
Order command	3	3	3	–
Writing	1	1.0 ± 0.00	0.98 ± 0.14	n.s
Naming	2	2	2	–
Naming	10	9.11 ± 1.02	9.92 ± 0.27	<0.001
Picture comprehension	4	4	4	–
Repeat words	2	1.94 ± 0.23	2.0 ± 0.00	n.s
Repeat phrase/sentences	2	2	2	–
Irregular words	1	0.33 ± 0.47	0.98 ± 0.14	<0.001
Verbal fluency	14	6.24 ± 2.14	9.33 ± 1.74	<0.001
Letter	7	2.15 ± 1.13	3.98 ± 1.55	<0.001
Animal	7	4.09 ± 1.27	5.35 ± 0.82	<0.001
Visual spatial &perceptual abilities	16	13.35 ± 2.52	15.45 ± 1.06	<0.001
Pentagon	1	0.59 ± 0.49	1.00 ± 0.00	<0.001
Cube	2	1.06 ± 0.85	1.84 ± 0.36	<0.001
Visuospatial	5	3.7 ± 1.47	4.61 ± 0.87	<0.001
Perceptual dots	4	4	4	–
Perceptual shapes	4	4	4	–
Total score	100	82.65 ± 6.91	93.73 ± 3.32	<0.001

Data were presented as mean ± SD.
ns: non-significant.

significantly higher than the control, and the effect studied was correlated with Al level and duration of employment. Moreover blood lead levels were measured in the exposed workers to exclude one possible confounder and none of the workers exceeded the permissible level. Additionally metal fraction other than Al in the total dust was about 31% which is less than the Al fraction alone. The individual metal levels were all much less than the permissible exposure levels set by the Egyptian EPA and these levels were for Cu, As, Zn, Pb, Cr, and Mn. Nonetheless we cannot exclude the role of these exposures and can only deduce that the effect studied (cognitive functions) was related mainly to Al exposure.

Addenbrooke's Cognitive Examination—Revised (ACE-R) test has been proposed as a simple and statistically robust tool for screening of cognitive impairment [63]. Although exposed workers did not present with signs or symptoms of neurological or neurobehavioral disorders they showed an inferior total score of ACE-R in comparison to the control group, which points to some degree of cognitive impairment. Mild cognitive impairment (MCI) is a heterogeneous disorder, and people suffering from it may progress to dementia or remain relatively stable and decline cognitively as in normal aging [30]. Among studies that investigated neurobehavioral changes in relation to occupational Al exposure, no study was found that evaluated cognitive functions using the ACE-R test. However, in the context of using the conventional MMSE, several studies demonstrated low scoring of MMSE among individuals exposed to Al, either environmentally in relation to increased Al content in the drinking water [64] or occupationally [19]. The neurological testing in these studies was not as comprehensive as in the Finnish study by Akila et al. but was consistent with their results as well as several others [65–67].

Every cognitive function screening tool is typically judged by its ability to accurately distinguish between those with and those without dementia, on the basis of cut-off scores. Crawford and co-worker made a systematic review on the accuracy and clinical utility of the ACE-R test in the diagnosis of dementia and they identified different cut-off scores. They chose the previously set 88 cutoff score as it seemed able to distinguish well between those with and those without cognitive impairment (sensitivity = 0.94, specificity = 0.89) [63,68]. However, likelihood ratios of dementia were generated for scores between 88 and 82 [68]. Unfortunately, we could not find any cognitive screening study that is comparable to ours regarding the age of the participants

Table 4
Comparison of serum Al, APP, cathepsin D activity and ACE-R test score in both exposed subgroups (based on median level = 83.5 of ACE-R test score) and control group.

Variable	Exposed subgroup above median N = 25 (46.2%)	Exposed subgroup below median N = 29 (53.7%)	Control N = 51
ACE-R score	88.84 ± 3.15(a)*(b)*	77.31 ± 4.26(a)*	93.73 ± 3.32(b)*
Serum Al (μg/L)	17.28 ± 6.59(a)*(b)*	22.86 ± 11.10(a)*	4.43 ± 2.17(b)*
Serum APP (ng/mL)	96.44 ± 15.36(a)*	99.31 ± 18.54(a)*	40.69 ± 8.79(b)*
Serum cathepsin D activity (RFU)	16.88 ± 6.88(a)*	15.22 ± 6.75(a)*	24.30 ± 5.01(b)*

Results are presented as mean ± SD.
(a) Values differ significantly from control.
(b) Values differ significantly from exposed subgroup below median of ACE-R test score.
ACE-R = Addenbrooke's Cognitive Examination – Revised; APP = amyloid precursor protein, RFU = relative fluorescence unit.
* Denotes significance (P < 0.001).

Table 5
Correlation between age, duration of work and total ACE-R test score with serum Al, APP and cathepsin D activity among exposed group.

		Serum Al (μg/L)	APP (ng/mL)	Cathepsin D activity (RFU)	Total ACE-R
Age	r	0.248	0.393	0.059	−0. 308
	P	n.s	0.003**	n.s	0.024*
Duration of work	r	0.349	0.416	−0.048	−0. 391
	P	0.01**	0.002**	n.s	0.003**
Total ACE-R	r	−0.317	−0.082	−0.02	–
	P	0.02*	n.s	n.s	
APP	r	0.365	–	−0. 558	−0.082
	P	0.007**		<.001**	ns
Cathepsin D activity	r	−0. 274	−0.558	–	−0.02
	P	0.045*	<.001**		ns

ns: statistically nonsignificant.
* Statistically significant ($P < 0.05$).
** Statistically significant ($P < 0.01$).

or the working circumstances that carry the risk of Al exposure. Additionally, there are no Egyptian norms for ACE-R among different age or educational level categories. Therefore, we could not apply any of the mentioned cut-off scores. However, the median total score of the ACE-R test in exposed workers was found to be 83.5 which was close to several previously set cut-off scores.

Memory, language naming, verbal fluency (reflect executive functions & verbal memory) and visuospatial abilities were significantly affected in exposed workers compared with their referent controls. Memory affliction probably reflects damage to the hippocampus where there is a high content of Al [69]. Akila et al. reported poor performance in the memory for designs and in more difficult block design items demanding preliminary visuospatial analysis among inert gas welders exposed to aluminum [65]. Experimentally, memory and spatial learning defects were reported in rats exposed to Al in diet [70]. In their meta-analysis study, Meyer-Baron and co-workers recommended neurobehavioral studies on implicit and explicit memory, visuo-spatial processing and central odor processing as they seem the most appropriate way to answer questions about functional impairments in occupational settings and to address the most distinctly affected brain areas [71]. These studies provided evidence of some degree of cognitive impairment in workers exposed to Al as measured in their serum. However, this issue is unresolved since several conflicting reports exist [20–22,57,72].

In this study exposed workers displayed overexpression of serum APP. Moreover, APP significantly positively correlated with both duration of work and serum Al. Al involvement in amyloidogenesis had been investigated and indeed strong evidence supports Al as an important factor in initiating the formation of amyloid plaques [4]. Up-regulated expression of APP occurs early in the cascade of events that leads to amyloid plaque formation in the human brain. Walton and Wang reported up-regulation of APP expression in brain tissues induced by chronic exposure to human relevant levels of dietary Al through generation of reactive oxygen species [29]. Over-expression of APP in hippocampal neurons leads to elevated Aβ peptide production, subsequent depression of excitatory transmission and disruption of both presynaptic and postsynaptic compartments [73]. Li and coworkers demonstrated the interaction between Al and the generation of Aβ peptide. Al accelerated Aβ peptide formation in mouse brains via oxidative stress. Further, vitamin E, as an anti-oxidant agent, reversed the effect of Al on lipid peroxidation and Aβ peptide formation [74,75]. Alterations of APP in platelets in AD patients were reported by two groups [76,77]. Sensitivities and specificities for AD diagnosis were in the 80–95% range, based on post hoc cutoff scores. The level of APP isoform ratio correlated with disease severity and progression. Less invasive procedures for biomarker ascertainment are highly desirable. Indeed APP in CSF and blood are promising novel biomarkers of AD [24,30].

In physiologically normal metabolism, Aβ peptide levels appear to be strictly regulated, resulting in a low level of Aβ peptide and no deposition in the brain. Recent reports indicate that Aβ peptide is taken up predominantly by microglia via class A and class B scavenger receptors. Then, the internalized Aβ peptides accumulate and are degraded in the lysosomes of microglia by CD enzyme [47].

Disturbance of the normal balance and extracellular localization of cathepsins may contribute to neurodegeneration [46]. Urbanelli et al. deduced an altered balance of CD in skin fibroblasts from patients affected either by sporadic or familial forms of AD which reinforced the hypothesis that a lysosomal impairment may be involved in AD pathogenesis and can be detected not only in the CNS but also at a peripheral level [78]. From the results reported herein it appears that CD balance was altered in the plasma of exposed workers with statistically significant decrements in the CD activity compared to the referent control. This may be considered as a toxic feature of Al, and a possible mechanism for amyloidogenesis. Indeed quantitative and qualitative disturbances of CD were reported in AD patients' blood lymphocytes compared to healthy donors. Furthermore, CD seamed to undergo an extralysosomal redistribution and was released in the plasma [79]. However, contradictory to these results Menéndez-González et al. studied the activity of serum CD in different stages of AD as well as in patients with MCI and vascular dementia. Their results did not support CD activity as a useful biomarker for dementias since they found no significant differences between AD stages or between AD and MCI or VD [80].

Regarding the relation between amyloidogenesis markers (APP level and CD activity) and cognitive functions (total ACE-R test score), no statistically significant difference between both parameters was found in the exposed subgroups of the higher and lower scores. Moreover no statistically significant correlation was found between these parameters and the total ACE-R score. Therefore despite the link between Al exposure in this study with cognitive impairment and amyloidogenesis, Al exposure-associated cognitive dysfunction probably does not relate to amyloidogenesis. Indeed in spite of the numerous efforts and the accumulating evidence in neurotoxicology research, the mechanisms of Al neurotoxicity are still not completely elucidated. Apart from amyloidogenesis, the involvement of oxidative stress, membrane biophysics alterations, deregulation of cell signaling and the impairment of neurotransmission were suggested as possible mechanisms of Al neurotoxicity [81].

5. Conclusion

Occupational exposure to Al, mainly in Al smelters, is associated with cognitive impairment. Our study provides evidence in support of the effect of Al exposure in occupational setting on APP and CD activity whose role in amyloidogenesis was previously proven. However the non-significant correlation between ACE-R test score and APP or CD activity does not support the role of amyloidogenesis as one mechanism of cognitive impairment and limits the usefulness of these potential biomarkers as screening tools for cognitive function in susceptible individuals. These results may be attributed to rather small sample size, the cross sectional design of the study, the multifactorial nature of Al neurotoxicity, together with the occupational nature of exposure to Al and the

possible added role of other exposures in the workplace that were not completely or efficiently controlled. Further studies are needed to investigate these deductions.

Abbreviations

Al	aluminum
AD	Alzheimer's disease
NFTs	neurofibrillary tangles
Aβ	peptide amyloid β peptide
DAE	dialysis associated encephalopathy
MMSE	Mini Mental State Exam
CSF	cerebrospinal fluid
CD	cathepsin D
t-tau	total tau protein
P-tau	phosphorylated tau protein
Aβ42	amino acid isoform of A β
ACE-R	Addenbrooke's Cognitive Examination — Revised
OSHA	Occupational Safety and Health Administration
MCI	mild cognitive impairment

Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jinorgbio.2014.06.003.

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