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Serum zinc is decreased in Alzheimer's disease and serum arsenic correlates positively with cognitive ability

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Abstract Zinc, copper, and iron aggregate Aβ and accumulate in Alzheimer's disease (AD) plaques. Some metals are increased in AD vs. control serum. The authors examined levels of 12 metals in serum of 44 AD and 41 control subjects. Zinc decreased from 12.3 to 10.9 μmol/L (means, $p = 0.0007$). Arsenic positively correlated with Mini-Mental State Examination score ($p < 0.0001$). Zinc deposition in brain amyloid might deplete zinc from other body compartments, such as serum. The arsenic correlation might be caused by the major contribution of seafood consumption to intake of both arsenic and docosahexaenoic acid, of which the latter may delay AD.

Keywords Alzheimer's disease · Aluminum · Arsenic · Metals · MMSE · Serum · Zinc

Introduction

Metals may play an important role in Alzheimer's disease (AD). Amyloid plaques in AD brain contain

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Aβ and metals, including zinc, copper, and iron (Bush and Tanzi 2002; Deibel et al. 1996; Religa et al. 2006; Smith et al. 1997). Ions of zinc, copper, and iron can induce Aβ peptide aggregation (Bush and Tanzi 2002). However, it is not yet clear what role these metals play in the chain of cause and effect in AD; i.e., whether they cause changes, either harmful or beneficial, in Aβ peptide aggregation or biochemical properties in vivo, or whether these ions accumulate after plaques have already been deposited. Except for one recent, very large study (Gerhardsson et al. 2008), previous studies have measured metal concentrations in small numbers (10 or fewer) of AD brains (Deibel et al. 1996; Religa et al. 2006). To obtain more insight into metal alterations in AD, larger sample numbers would be desirable.

Advantages of serum samples, as compared to brain specimens, are that serum can be taken from a greater number of patients, and from patients at various stages of AD. A disadvantage of serum is that it does not provide direct information on the metal status of the brain. However, AD serum has been reported to contain altered concentrations of some metals. Manganese concentration was greater in plasma of AD than of controls, but the reverse was true in cerebrospinal fluid, suggesting a relation between the brain and blood levels of at least some metals (Gerhardsson et al. 2008). Higher serum levels of copper (Gonzalez et al. 1999; Smorgon et al. 2004; Squitti et al. 2006) were reported in AD than controls, and were associated with faster progression of dementia (Squitti et al. 2009). Studies have also found increased serum aluminum concentration in AD (Roberts et al. 1998; Zapatero et al. 1995), which might be due to greater absorption of dietary aluminum (Taylor et al. 1992). To examine the relation of metals to AD, we measured serum total levels of 12 elements in 44 AD and 41 control subjects.

Materials and methods

Subjects were ethnic Chinese (as determined by the investigators) living in Hong Kong. Alzheimer's disease (AD) patients ($n = 44$) had NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association) diagnosis of probable or possible AD (McKhann et al. 1984), and were recruited from geriatric outpatient clinics ($n = 35$) and old age homes ($N = 9$). Control subjects ($n = 41$) lacked neurological disease. The Cantonese Mini-Mental Status Examination (MMSE, maximum score 30; Chiu et al. 1994) was administered to 43 AD and one control subject. The local Clinical Research Ethics Committees approved study of these subjects. All subjects gave written informed consent, except that in AD patients who could not give consent, surrogate consent was obtained from their guardians. Serum was collected from all subjects.

Metallic and non-metallic trace elements (aluminum, arsenic, beryllium, chromium, cobalt, copper, iodine, iron, manganese, nickel, selenium, and zinc) in serum samples were simultaneously assayed by inductively coupled plasma-mass spectrometry (ICP-MS 7500c, Agilent Technologies Inc, Palo Alto, CA, USA). Samples were pre-treated with a diluent containing 0.05% tetra-methyl ammonium hydroxide and a mixture of internal standards containing rhodium, yttrium and iridium before analysis. An on-line reaction cell filled with helium was used to eliminate polyatomic interference due to compounds with similar mass-charge ratio. The inter-assay coefficients of variation were generally <9%. The detection limit of aluminum, arsenic, beryllium, chromium, cobalt, copper, iodine, iron, manganese, nickel, selenium, and zinc were 0.025 μmol/L, 2 nmol/L, 0.5 nmol/L, 3 nmol/L, 0.24 nmol/L, 6 nmol/L, 0.03 μmol/L, 4.1 nmol/L, 2.4 nmol/L, 2.3 nmol/L, 0.08 μmol/L and 0.01 μmol/L, respectively. Beryllium and nickel concentrations for many samples were below the detection limits, thus we did not analyze these metals.

Statistical calculations were performed using SPSS 14.0 software (SPSS, Chicago, Illinois, USA). Significance of differences in continuous variables between AD and controls was determined by testing for normality of distributions using the Kolmogorov–Smirnov test with Lilliefors significance level, and then using 2-sided independent sample t tests for normal distributions and Mann–Whitney tests otherwise. Of the metals, only zinc was normally distributed. All other metals were compared using the Mann–Whitney test and natural log transformed for the logistic regression models.

Results

Table 1 shows descriptive statistics and unadjusted and adjusted p-values comparing metal concentrations for AD and control groups. Figure 1 displays metal concentrations for AD and control groups. In the unadjusted comparisons, the AD patients had significantly lower levels of iron, zinc, iodine, and chromium, and significantly higher levels of aluminum. If a Bonferroni adjusted significance level of $0.05/10 = 0.005$ was used, then only zinc was significantly different. After adjustment for age and sex (model 1), iron, zinc, and aluminum significantly differed, with zinc and aluminum still differing after Bonferroni adjustment. After adjustment for all metals simultaneously (model 2), zinc, selenium, and chromium significantly differed, with zinc and selenium differing after Bonferroni adjustment. Of five metals in the forward stepwise regression (model 3), zinc was significant at $p < 0.005$. Zinc significantly differed in all models.

MMSE scores were significantly correlated with aluminum (Pearson's correlation coefficient: -0.33, $p = 0.03$) and arsenic (Fig. 2). Arsenic remained significant after Bonferroni adjustment.

Discussion

In most carefully designed studies, zinc concentration increased in brain tissue or cerebrospinal fluid of AD compared to controls (Capasso et al. 2005; Cornett et al. 1998; Deibel et al. 1996; Religa et al. 2006). In serum by contrast, the literature generally reported either no change (Dong et al. 2008; Gerhardsson et al. 2008; Gonzalez et al. 1999; Ozcankaya and Delibas 2002) or a decrease (this study) in AD compared to controls. This contrast was supported by a report of a significant negative correlation between amyloid plaque numbers and serum zinc concentration in AD (Tully et al. 1995). Brain zinc levels positively correlated with levels of Aβ peptide (which forms amyloid plaques), plaque numbers, and dementia severity in AD (Religa et al. 2006). Zinc is a potent inducer of Aβ aggregation (Bush and Tanzi 2002), thus the decrease we observed in serum zinc level in AD might be due to deposition and sequestration of zinc in brain amyloid, perhaps depleting zinc in other body compartments. Zinc released from the neocortical glutamatergic synapse is in communication with the plasma (Friedlich et al. 2004). Therefore, levels of plasma zinc may partly reflect circulation from

Table 1 Differences between AD and control subjects

	Controls ($n = 41$)	AD ($n = 44$)	Unadj. (p)	Model 1 (p)	Model 2 (p)	Model 3 (p)
Age (year)[a]	79.1 ± 6.0	74.3 ± 8.7	.0041		.027	.0056
Women/men (n)[b]	20/21	29/15	.13		.0030	.0006
Metals (nmol/L)						
Iron	23,800 ± 11,000	17,700 ± 8,200	.017	.037	.18	
Copper	15,300 ± 2,700	16,200 ± 3,500	.23	.37	.72	
Zinc[a]	12,300 ± 1,600	10,900 ± 1,600	.0001	.0007	.0015	<.0001
Selenium	1,390 ± 240	1,420 ± 230	.56	.29	.0020	.0078
Iodine	610 ± 170	560 ± 140	.038	.069	.058	.032
Aluminum	580 ± 620	905 ± 630	.0055	.0023	.12	.0077
Arsenic	38.7 ± 37	35.7 ± 40	.57	.61	.096	
Cobalt	23.5 ± 28	9.1 ± 12	.27	.55	.64	
Chromium	17.2 ± 12	22.7 ± 21	.032	.093	.025	.020
Manganese	13.3 ± 9.2	21.4 ± 21	.065	.26	.48	

Results are presented as median ± interquartile range and unadjusted (Unadj.) p value for Mann–Whitney test, except where indicated by, [a] which indicates mean ± SD and unadjusted p value for t-test, or by, [b] which indicates Fisher's exact test. Adjusted p-values were calculated using logistic regression with case–control status as the outcome variable. Three types of models were fit: (1) one metal at a time with age and sex as additional control covariates; (2) all metals in one model along with age and sex; and (3) a model with variables selected from model (2) by forward stepwise selection ($p = .05$, likelihood ratio test)

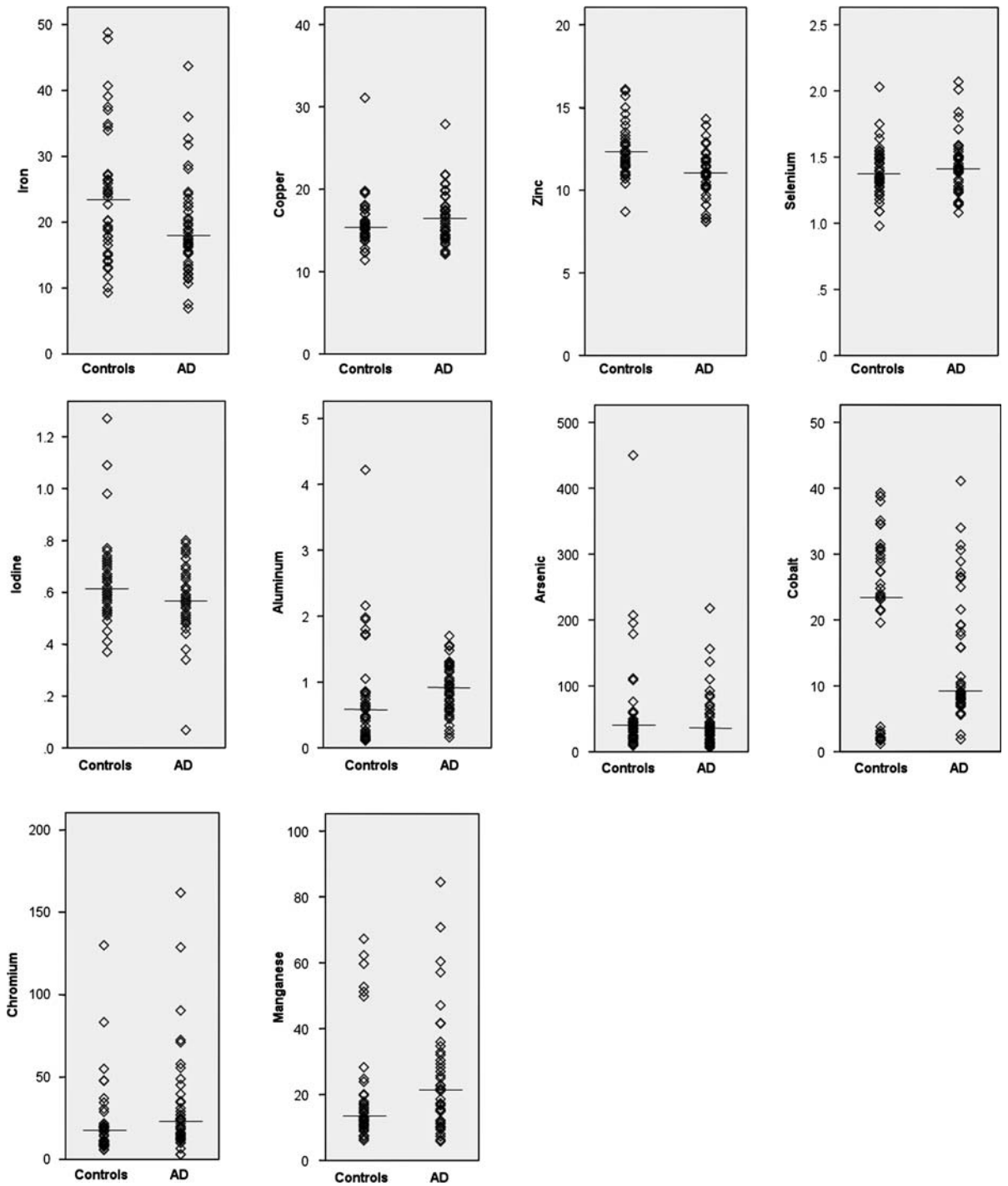

Fig. 1 Scatterplot of serum metal concentrations for control or AD subjects (μmol/L, except for arsenic, cobalt, chromium, and manganese, which are in nmol/L). *Bars* indicate medians (except for zinc, which are means)

synapses, where accumulated Aβ traps zinc, decreasing plasma zinc. This notion was supported by a study in which plasma zinc level in AD was less than the level in controls but rose significantly and into the normal range after treatment with the zinc-binding compound clioquinol, suggesting that the treatment

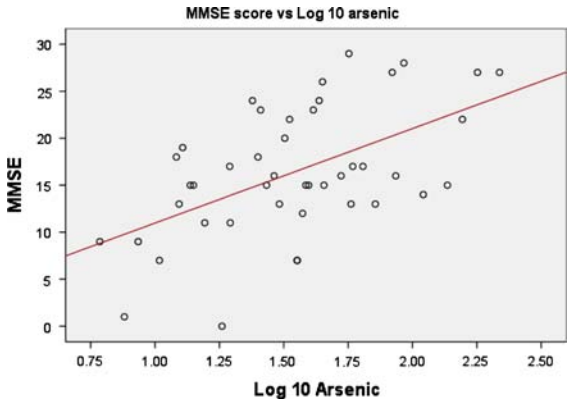

Fig. 2 Two-way scatterplot of MMSE score vs. logarithm of arsenic concentration (nmol/L). Pearson's correlation coefficient for the association is 0.55 ($p < 0.0001$)

broke down amyloid in the synapse, allowing the synaptic zinc to resume its contribution to plasma (Ritchie et al. 2003). The lack of difference in zinc level between AD and control serum in two other studies (Gonzalez et al. 1999; Ozcankaya and Delibas 2002), in contrast to our very significant difference, remains unexplained.

Another possible cause of decreased serum zinc in AD may be diet. A study of nutrient intake found that severe AD patients (institutionalized, with a mean MMSE score of 3) consumed less zinc than controls, probably due to a generally poor diet (Tabet et al. 2001). But zinc intake of mild/moderate AD patients living at home did not differ from controls (Tabet et al. 2001). The unadjusted difference in serum zinc between our AD and control subjects remained significant when we excluded from analysis either institutionalized AD patients ($p = 0.001$) or subjects with an MMSE score < 10 ($p = 0.002$), suggesting that deficient zinc intake in severe AD may not be the cause of our observed difference in serum zinc between AD and controls.

In contrast to zinc, aluminum concentration tended to decrease or remain unchanged in the brains of AD patients compared to those of control subjects (Bjertness et al. 1996; Religa et al. 2006), but to increase in AD serum (Roberts et al. 1998; Zapatero et al. 1995), as we found. The increase in serum level may be due to more efficient uptake of dietary aluminum in AD, but the cause of this change in uptake is unknown (Miu et al. 2004; Roberts et al. 1998).

An involvement of arsenic in AD has been hypothesized (Gharibzadeh and Hoseini 2008). However, our results show no significant association of serum arsenic level with AD. We did find a strong positive correlation of serum arsenic concentration with MMSE score. There are several possible explanations for this finding. As with zinc, arsenic might be absorbed by amyloid plaques, depleting arsenic from other body compartments, such as blood. Thus, more severe AD with more plaques may show less serum arsenic. However, no studies have yet been published on arsenic binding to Aβ or associating with amyloid plaques.

Polymorphisms in glutathione S-transferase omega genes GSTO1 and GSTO2 were associated with the age of onset of AD (Li et al. 2003). These polymorphisms might exert their effects through changes in expression or function of the GSTO1 or GSTO2 enzymes. For example, GSTO1 expression differed between hippocampus of AD and controls (Li et al. 2003), and the delE155 form of GSTO1 exhibited specific monomethylarsonate activity double that of wild-type GSTO1 (Schmuck et al. 2005). The arsenic metabolic activity of GSTO1 suggests that polymorphisms may affect both AD onset age and arsenic metabolism. This might explain the correlation of MMSE scores with arsenic levels. However, age at testing (we do not have age of onset data) did not correlate with either arsenic level ($p = 0.49$) or MMSE score ($p = 0.36$) in the 44 subjects shown in Fig. 2. In addition, arsenic levels did not significantly differ between AD and control subjects. Besides arsenic compounds, GSTO1 and GSTO2 act on many substrates relevant in AD, including interleukin-1β and ascorbate, and the polymorphisms may affect AD age of onset via actions on any of these other substrates rather than on arsenic compounds (Schmuck et al. 2005).

Finally, most arsenic enters the human body through eating seafood (except in areas with very low seafood consumption or unusually high concentrations of arsenic in drinking water), thus serum arsenic concentration may often be an indirect measure of seafood consumption (Tao and Bolger 1999). High intake of inorganic arsenic, such as from drinking water, can impair cognition, but seafood arsenic is generally in organic, non-toxic forms (Tao and Bolger 1999). Eating fish is associated with reduced AD risk, and the association of high serum arsenic with high MMSE score may be due to seafood consumption (Morris et al. 2003). High *n* -3 fatty acid consumption, particularly of docosahexaenoic acid,

of which seafood is a rich source, is associated with reduced AD risk and might be responsible for the association of seafood and AD (Morris et al. 2003). Curiously, although arsenic was strongly associated with MMSE score, we saw no decrease in arsenic in AD as compared to controls. Dietary *n* -3 fatty acid consumption increased learning and memory in animal and human studies (Chen et al. 2006; Chiu et al. 2008; Morris et al. 2003), therefore it might be that docosahexaenoic acid improves cognition in humans who are developing AD but whose cognition has not yet deteriorated enough to be diagnosed, thus delaying the age of onset of AD and reducing the incidence of AD in prospective studies (Morris et al. 2003) but not affecting the risk of eventually developing AD in case–control studies such as ours.

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References

Bjertness E, Candy JM, Torvik A, Ince P, McArthur F, Taylor GA, Johansen SW, Alexander J, Gronnesby JK, Bakketeig LS, Edwardson JA (1996) Content of brain aluminum is not elevated in Alzheimer disease. Alzheimer Dis Assoc Disord 10:171–174

Bush AI, Tanzi RE (2002) The galvanization of beta-amyloid in Alzheimer's disease. Proc Natl Acad Sci U S A 99: 7317–7319

Capasso M, Jeng JM, Malavolta M, Mocchegiani E, Sensi SL (2005) Zinc dyshomeostasis: a key modulator of neuronal injury. J Alzheimers Dis 8:93–108

Chen CC, Chaung HC, Chung MY, Huang LT (2006) Menhaden fish oil improves spatial memory in rat pups following recurrent pentylenetetrazole-induced seizures. Epilepsy Behav 8:516–521

Chiu HFK, Lee HC, Chung WS, Kwong PK (1994) Reliability and validity of the Cantonese version of the mini-mental state examination—a preliminary study. J Hong Kong Coll Psychiatrists 4(Suppl. 2):25–28

Chiu CC, Su KP, Cheng TC, Liu HC, Chang CJ, Dewey ME, Stewart R, Huang SY (2008) The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry 32:1538–1544

Cornett CR, Markesbery WR, Ehmann WD (1998) Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain. Neurotoxicology 19:339–345

Deibel MA, Ehmann WD, Markesbery WR (1996) Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. J Neurol Sci 143:137–142

Dong J, Robertson JD, Markesbery WR, Lovell MA (2008) Serum zinc in the progression of Alzheimer's disease. J Alzheimers Dis 15:443–450

Friedlich AL, Lee JY, van GT, Cherny RA, Volitakis I, Cole TB, Palmiter RD, Koh JY, Bush AI (2004) Neuronal zinc exchange with the blood vessel wall promotes cerebral amyloid angiopathy in an animal model of Alzheimer's disease. J Neurosci 24:3453–3459

Gerhardsson L, Lundh T, Minthon L, Londos E (2008) Metal concentrations in plasma and cerebrospinal fluid in patients with Alzheimer's disease. Dement Geriatr Cogn Disord 25:508–515

Gharibzadeh S, Hoseini SS (2008) Arsenic exposure may be a risk factor for Alzheimer's disease. J Neuropsychiatry Clin Neurosci 20:501

Gonzalez C, Martin T, Cacho J, Brenas MT, Arroyo T, Garcia-Berrocal B, Navajo JA, Gonzalez-Buitrago JM (1999) Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. Eur J Clin Invest 29:637–642

Li YJ, Oliveira SA, Xu P, Martin ER, Stenger JE, Scherzer CR, Hauser MA, Scott WK, Small GW, Nance MA, Watts RL, Hubble JP, Koller WC, Pahwa R, Stern MB, Hiner BC, Jankovic J, Goetz CG, Mastaglia F, Middleton LT, Roses AD, Saunders AM, Schmechel DE, Gullans SR, Haines JL, Gilbert JR, Vance JM, Pericak-Vance MA, Hulette C, Welsh-Bohmer KA (2003) Glutathione S-transferase omega-1 modifies age-at-onset of Alzheimer disease and Parkinson disease. Hum Mol Genet 12:3259–3267

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. Neurology 34:939–944

Miu AC, Olteanu AI, Miclea M (2004) A behavioral and ultrastructural dissection of the interference of aluminum with aging. J Alzheimers Dis 6:315–328

Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, Aggarwal N, Schneider J (2003) Consumption of fish and *n* -3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 60:940–946

Ozcankaya R, Delibas N (2002) Malondialdehyde, superoxide dismutase, melatonin, iron, copper, and zinc blood concentrations in patients with Alzheimer disease: cross-sectional study. Croat Med J 43:28–32

Religa D, Strozyk D, Cherny RA, Volitakis I, Haroutunian V, Winblad B, Naslund J, Bush AI (2006) Elevated cortical zinc in Alzheimer disease. Neurology 67:69–75

Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, Kiers L, Cherny R, Li QX, Tammer A, Carrington D, Mavros C, Volitakis I, Xilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, Masters CL (2003) Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. Arch Neurol 60:1685–1691

Roberts NB, Clough A, Bellia JP, Kim JY (1998) Increased absorption of aluminium from a normal dietary intake in dementia. J Inorg Biochem 69:171–176

Schmuck EM, Board PG, Whitbread AK, Tetlow N, Cavanaugh JA, Blackburn AC, Masoumi A (2005) Characterization of the monomethylarsonate reductase and dehydroascorbate reductase activities of Omega class glutathione transferase variants: implications for arsenic metabolism and the age-at-onset of Alzheimer's and Parkinson's diseases. Pharmacogenet Genomics 15:493–501

Smith MA, Harris PL, Sayre LM, Perry G (1997) Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A 94:9866–9868

Smorgon C, Mari E, Atti AR, Dalla NE, Zamboni PF, Calzoni F, Passaro A, Fellin R (2004) Trace elements and cognitive impairment: an elderly cohort study. Arch Gerontol Geriatr Suppl 9:393–402

Squitti R, Barbati G, Rossi L, Ventriglia M, Dal FG, Cesaretti S, Moffa F, Caridi I, Cassetta E, Pasqualetti P, Calabrese L, Lupoi D, Rossini PM (2006) Excess of nonceruloplasmin serum copper in AD correlates with MMSE, CSF [beta]-amyloid, and h-tau. Neurology 67:76–82

Squitti R, Bressi F, Pasqualetti P, Bonomini C, Ghidoni R, Binetti G, Cassetta E, Moffa F, Ventriglia M, Vernieri F, Rossini PM (2009) Longitudinal prognostic value of serum "free" copper in patients with Alzheimer disease. Neurology 72:50–55

Tabet N, Mantle D, Walker Z, Orrell M (2001) Vitamins, trace elements, and antioxidant status in dementia disorders. Int Psychogeriatr 13:265–275

Tao SS, Bolger PM (1999) Dietary arsenic intakes in the United States: FDA Total Diet Study, September 1991–December 1996. Food Addit Contam 16:465–472

Taylor GA, Ferrier IN, McLoughlin IJ, Fairbairn AF, McKeith IG, Lett D, Edwardson JA (1992) Gastrointestinal absorption of aluminium in Alzheimer's disease: response to aluminium citrate. Age Ageing 21:81–90

Tully CL, Snowdon DA, Markesbery WR (1995) Serum zinc, senile plaques, and neurofibrillary tangles: findings from the Nun Study. Neuroreport 6:2105–2108

Zapatero MD, Garcia de JA, Pascual F, Calvo ML, Escanero J, Marro A (1995) Serum aluminum levels in Alzheimer's disease and other senile dementias. Biol Trace Elem Res 47:235–240