

# Altered ionized magnesium levels in mild-to-moderate Alzheimer's disease

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**Abstract.** Magnesium deficiency is present in several chronic, age-related diseases, including cardiovascular, metabolic and neurodegenerative diseases. Alzheimer's disease (AD) is the most common cause of dementia. The aim of the present study was to study magnesium homeostasis in patients with mild to moderate AD. One hundred and one elderly ( $\geq 65$  years) patients were consecutively recruited (mean age:  $73.4 \pm 0.8$  years; M/F: 42/59). In all patients, a comprehensive geriatric assessment was performed including cognitive and functional status. Admission criteria for the AD group (diagnosed according to the DSM-IV and the NINCDS-ADRDA criteria) included: mild to moderate cognitive impairment (MMSE score: 11–24/30, corrected for age and education). Blood samples were analyzed for serum total magnesium (Mg-tot) and serum ionized magnesium (Mg-ion). AD patients had significantly lower MMSE scores ( $20.5 \pm 0.7$  vs  $27.9 \pm 0.2$ ;  $p < 0.001$ ), and for the physical function tests. Mg-ion was significantly lower in the AD group as compared to age-matched control adults without AD ( $0.50 \pm 0.01$  mmol/L vs  $0.53 \pm 0.01$  mmol/L;  $p < 0.01$ ). No significant differences were found in Mg-tot between the two groups ( $1.91 \pm 0.03$  mEq/L vs  $1.95 \pm 0.03$  mEq/L;  $p = \text{NS}$ ). For all subjects, Mg-ion levels were significantly and directly related only to cognitive function (Mg-ion/MMSE  $r = 0.24$   $p < 0.05$ ), while no significant correlations were found in this group of patients between magnesium and ADL or IADL. Our results show the presence of subclinical alterations in Mg-ion in patients with mild to moderate AD.

**Key words:** magnesium, Alzheimer, dementia, aging, ionized magnesium, ions

Magnesium deficiency has been related to several chronic, age-related diseases, including cardiovascular, metabolic and neurodegenerative diseases [1]. Alzheimer's disease (AD) is the most common cause of dementia in older populations, its incidence increasing dramatically with age [2].

AD is a progressive, neurodegenerative disorder characterized by cognitive and memory deterioration, progressive impairment of activities of daily living (ADL), and a variety of neuropsychiatric and behavioral disturbances [3]. The neuropatho-

logical hallmark of the disease is the presence of neurofibrillary tangles and amyloid plaques, impaired synaptic function, and cell loss [3]. The etiopathogenesis of AD remains unclear. There are ongoing efforts to elucidate the biochemical processes involved in the etiopathogenesis of AD. The role of magnesium in dementia and other degenerative disorders has been the focus of increased attention in recent years. Magnesium insufficiency and its altered concentrations in the brain, as well as the effects of magnesium supplementation in AD, have been investigated. Magnesium levels were found to be decreased in plasma and in various tissues of patients with Alzheimer's disease in clinical, experimental and autopsy studies [4-7].

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Magnesium is essential because of its role in more than 300 intracellular enzyme systems. Its concentration affects many biochemical mechanisms, including the NMDA-receptor response to excitatory amino acids [8, 9], cell membrane fluidity and stability [10], and the toxic effects of calcium [11].

Mg-ion is a precise and relatively easy tool with which to diagnose subclinical magnesium depletion in the aging populations [12]. However, measurement of Mg-ion levels has not yet been reported in patients with dementia. The aim of the present study was to study Mg-ion homeostasis in patients with mild to moderate AD.

## Subjects and methods

One hundred and one elderly ( $\geq 65$  years) patients (mean age:  $73.4 \pm 0.8$  years; M/F: 42/59; *table 1*), were consecutively recruited from those attending the Outpatient Clinic of the Geriatric Unit at the University Hospital of Palermo, Italy. Exclusion criteria included: severely altered general laboratory tests and/or not compensated acute disease, such as severe congestive heart failure, severe chronic obstructive pulmonary disease, angina pectoris, acute myocardial infarction or ictus in the six months prior to the study, severe uncontrolled hypertension ( $SBP \geq 180$  mmHg), uncontrolled diabetes mellitus, moderate to severe renal or hepatic disease, malnutrition. None of the recruited patients was receiving treatment

**Table 1.** Clinical characteristics of study subjects

| Parameter      | AD              | Control         |             |
|----------------|-----------------|-----------------|-------------|
|                | n=36            | n=65            |             |
| Age (years)    | $73.1 \pm 0.9$  | $73.8 \pm 1.1$  | NS          |
| M/F            | 15/21           | 27/38           | NS          |
| BMI            | $26.8 \pm 1.5$  | $28.0 \pm 1.6$  | NS          |
| SBP (mm Hg)    | $139 \pm 8$     | $141 \pm 7$     | NS          |
| DBP (mm Hg)    | $78 \pm 5$      | $79 \pm 5$      | NS          |
| HR (bpm)       | $72 \pm 5$      | $73 \pm 6$      | NS          |
| MMSE           | $20. \pm 0.7$   | $27.9 \pm 0.2$  | $p < 0.001$ |
| ADL            | $4.8 \pm 0.2$   | $5.4 \pm 0.13$  | $p < 0.001$ |
| IADL           | $4.9 \pm 0.4$   | $6.7 \pm 0.23$  | $p < 0.001$ |
| Mg-tot (mEq/L) | $1.91 \pm 0.03$ | $1.95 \pm 0.03$ | NS          |

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; ADL: activity of daily living, IADL: instrumental activity of daily living.

with diuretics or any other drugs that might have altered magnesium metabolism.

In all patients, a comprehensive geriatric assessment was performed, including cognitive and functional status. Cognitive and functional impairment was assessed using ADL [13], instrumental activities of daily living (IADL) [14], and mini-mental state examination (MMSE) tests [15]. The diagnosis of AD was consistent with the DSM-IV [16] and the NINCDS-ADRDA criteria for possible or probable AD [17]. Admission criteria in the AD group included: mild to moderate cognitive impairment (MMSE score: 11-24, corrected for age and education).

Subjects were fasted for at least 10 hours prior to the study. Blood samples were drawn and analyzed for serum total (Mg-tot) and serum ionized magnesium (Mg-ion). Mg-tot levels were measured using standard techniques with an automated chemistry analyzer (ISE 900/ISE 1800 modules of Modular Analytics SWA Roche Diagnostics Italia, Monza, Italy). Blood for Mg-ion was drawn into air-evacuated glass tubes containing an inter-cell separating matrix. After clotting and centrifugation, the tubes were inverted and serum was drawn off into a syringe anaerobically, the latter being capped and stored in a refrigerator freezer ( $0-4^{\circ}\text{C}$ ) for further analysis. An Mg ion-selective electrode (ISE) with a neutral, carrier-based membrane (Nova and Stat Profile 8 Ultra analyzers, Waltham, MA, USA) was used to measure serum Mg-ion [18, 19].

## Statistical analysis

All values are reported as the mean  $\pm$  SEM. Differences were assessed by paired t tests and with analysis of variance (ANOVA) for repeated measures. Differences were considered to be statistically significant for probability values lower than 0.05. Pearson's correlation coefficients were used to analyze linear correlations between variables. Statistical analyses were performed using Graph Pad version 4.0 software (Graph Pad Software, San Diego, CA, USA).

## Results

General characteristics of participants are described in *table 1*. No significant differences were

found among the groups as regards age, BMI, and systolic and diastolic blood pressure (*table 1*) and general laboratory values (data not shown).

As expected, AD patients had significant lower scores for MMSE ( $20.5 \pm 0.7$  versus  $27.9 \pm 0.2$ ,  $P < 0.001$ ), as well as for the physical function tests (ADL, IADL, *table 1*).

Mg-ion was significantly lower in the AD group versus age-matched older adults without AD ( $0.50 \pm 0.01$  mmol/L vs  $0.53 \pm 0.01$  mmol/L;  $p < 0.01$ , *figure 1*). No significant differences were found for Mg-T between the two groups ( $1.91 \pm 0.03$  mEq/L vs  $1.95 \pm 0.03$  mEq/L;  $p = \text{NS}$ , *table 1*). For all subjects, Mg-ion levels were significantly and directly related only to cognitive function (Mg-ion-MMSE,  $r = 0.24$ ;  $p < 0.05$ , *figure 2*), while no significant correlations were found in this group of patients between Mg-ion and ADL or IADL.

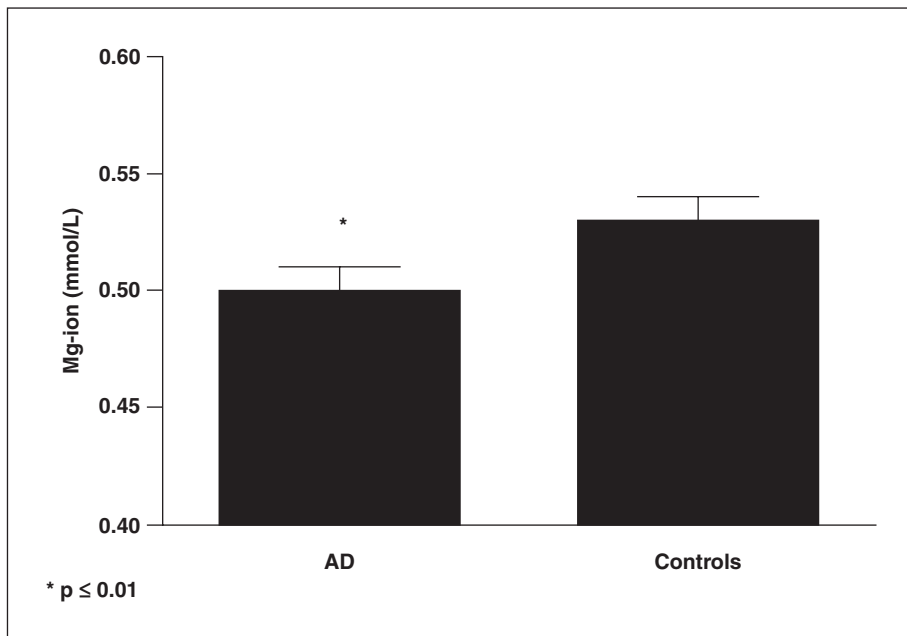
## Discussion

Our present results show the presence of alterations of magnesium levels in patients with AD.

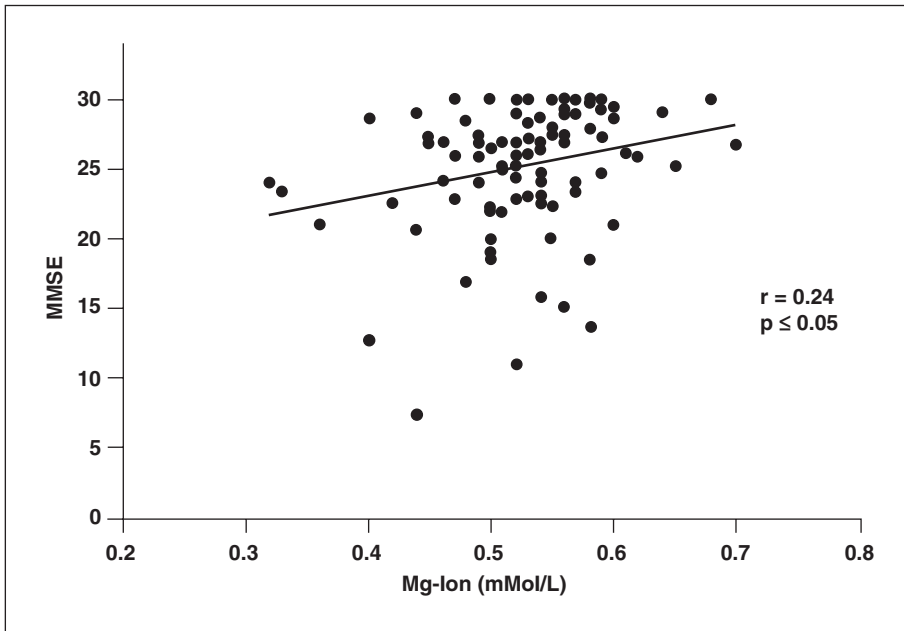
Lemke *et al.*, have previously reported decreased plasma Mg-tot values in patients with severe AD (mean MMSE  $5.1 \pm 2.6$ ) [7]. Our data extend this knowledge showing that already in patients with mild to moderate stage AD (mean MMSE  $20.5 \pm 0.7$ ), there is an alteration in magnesium homeostasis, revealed by a significant decrease of Mg-ion when compared to age-matched controls without AD. Our data also suggest that measurement of Mg-ion may be a better marker than Mg-Tot for diagnosing subclinical magnesium deficiency in AD subjects. Mg-ion levels were also significantly and directly related to cognitive function evaluated by MMSE.

Our group has previously used an ion-selective electrode (ISE), Mg-selective, to measure the active, ionized free magnesium (Mg-ion), and have suggested that this measurement is of help in detecting subclinical magnesium deficiency, in several age-related, clinical conditions, including diabetes mellitus, and hypertension [1, 12]. A close, direct relationship was found between Mg-ion and the intracellular magnesium measurement [19].

Aging and age-related diseases represent a major risk factor for magnesium deficiency [1].



**Figure 1.** Serum ionized magnesium levels (Mg-ion) in elderly subjects with Alzheimer's disease (AD), and in age-matched controls.



**Figure 2.** Relationship between serum ionized magnesium levels (Mg-ion) and cognitive function (MMSE).

The role of magnesium in dementia and other degenerative disorders has been the focus of increased attention in recent years. Magnesium levels were found to be decreased in various tissues of patients with AD in clinical, experimental and autopsy studies [4-7]. However, although previous studies have suggested a role for magnesium deficiency in AD, to our knowledge, this is the first report that has investigated alterations in Mg-ion in AD. In agreement with previous studies, in our study, plasma ionized magnesium levels were found to be decreased in AD.

Magnesium has been shown to have several effects on intellectual and neuronal functions *via* many of the biochemical mechanisms that are regulated by N-methyl-D-aspartate (NMDA) receptor response to excitatory stimuli, the stability of the cell membrane, and the toxic effects of calcium [5, 20]. Magnesium is directly involved in numerous important biochemical reactions because it is a necessary cofactor in over 300 intracellular enzyme reactions; more specifically, magnesium is necessary in all those processes that involve the use and transfer of adenosine triphosphate (ATP). Thus, magnesium is a critical

cofactor that regulates the activity of all enzymes involved in phosphorylation reactions, including tyrosine-kinase, as well as all other protein kinases, and all ATP and phosphate transfer-associated enzymes, such as the CaATPases in the plasma membrane and endoplasmic reticulum [1]. Magnesium deficiency may result in disorders of kinase activities. The activities of tyrosine kinase proteins have been reported to be significantly reduced in the AD hippocampus, and addition of 10 mM of magnesium to membrane fractions of hippocampal tyrosine kinase proteins are able to activate protein phosphorylation [21]. In the brain and fibroblast cultures obtained from AD patients, the translocation and activity of protein kinase C (PKC) was found to be defective [22], and both cytosolic and membrane-associated PKC responses involved in the regulation of amyloid precursor protein (APP) secretion, were reduced in frontal and temporal cortices, including the hippocampus of AD brains [22]. Downregulation of PKC is related to the memory impairment induced by chronic, intracerebroventricular infusion of beta-amyloid [23]. PKC down-regulation has also been reported to induce apoptosis *in*

*vitro* [24]. Magnesium is a potent neuroprotective agent against damage to synaptic transmission, and the mechanisms of magnesium neuroprotection with preservation of synaptic function may include altering the PKC response to an anoxic insult. Magnesium has been reported to induce the translocation of PKC to the membrane cytoskeleton and to augment its activity [25].

Oxidative stress and chronic inflammation may be another possible link between alterations in magnesium metabolism and AD. Inflammatory processes, particularly those mediating chronic inflammation, have been implicated as predictors or initiators of, or contributors to, chronic diseases and conditions primarily associated with aging, including AD. Increased oxidative stress and lipid peroxidation have been identified in AD [26-28]. Although the literature provides evidence connecting inflammation or inflammatory mediators with AD, the underlying biology linking mediators of inflammation with AD is unclear. Magnesium deficiency may be, at least, one of the physiopathological links that may help to explain the interactions between inflammation and oxidative stress, and AD.

Hypomagnesemia has been associated with inflammation and increased production of free oxygen radicals. Poor magnesium status may trigger the development of a proinflammatory state, but the sequence of events leading to the inflammatory response remains unclear. The mechanisms that may explain the proinflammatory effect of magnesium deficiency includes a stimulation of the production and thus increased circulating levels of inflammatory cytokines. In animals, several studies have shown that magnesium deprivation causes excessive production and release of proinflammatory molecules such as tumor necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , IL-6, vascular cell adhesion molecule (VCAM)-1, and plasminogen activator inhibitor (PAI)-1; increased circulating inflammatory cells (leukocyte and macrophage activation), and an increased hepatic production and release of acute phase proteins (i.e. complement,  $\alpha$ 2-macroglobulin, fibrinogen) [29-32].

Magnesium deficiency has also been associated, both in experimental animal models and in humans, with increased oxidative stress and decreased antioxidant defense [33-35]. Magnesium may also prevent oxygen radical formation by scavenging free radicals and by inhibiting xanthine oxidase and nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase [35]. Magnesium deficiency may decrease membrane integrity and membrane function, increasing susceptibility to oxidative stress and aging-related diseases [1].

Because magnesium acts as a physiological calcium antagonist, the modulation of intracellular calcium concentrations may also modify the NMDA-receptor response to excitatory amino acids, which, in turn, may contribute to neural degeneration [36]. The above-mentioned reasons, confirm that the availability of an adequate quantity of magnesium is a critical factor for normal neural cellular and tissue homeostasis.

Administration of magnesium has been suggested to be a strategy for reducing the damaging consequences of calcium-induced neuroinflammation in degenerative neurological disorders such as AD [10].

Recently, it has been shown that increasing brain magnesium leads to the enhancement of learning abilities, working memory, and short- and long-term memory in young and older rats [9]. Functionally, magnesium increased the number of functional, presynaptic release sites. Magnesium has been shown to induce an upregulation of NMDA receptors and its downstream signaling, and enhance synaptic plasticity [8].

Future studies should include follow-up measurements under controlled, dietary conditions, measurement of intracellular magnesium concentrations, and evaluation of intracellular free magnesium. Further studies are also needed to investigate the possibility that decreased Mg-ion levels correlate directly with inflammatory cytokines, and/or PKC activity, and/or oxidative stress determinants in the same patients, and to establish if lower Mg-ion levels actually lead to the development of AD, or if they are simply a consequence of the disease. Also, the possibility that maintaining an optimal magnesium balance throughout life might help in preventing or significantly retarding the manifestations of chronic diseases, including AD, is an hypothesis that needs to be tested in future, prospective studies.

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## Disclosure

None of the authors has any conflict of interest or financial support to disclose.

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