Efficacy of chelation therapy to remove aluminium intoxication

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ABSTRACT

There is a distinct correlation between aluminium (Al) intoxication and neurodegenerative diseases (ND). We demonstrated how patients affected by ND showing Al intoxication benefit from short-term treatment with calcium disodium ethylene diamine tetraacetic acid (EDTA) (chelation therapy). Such therapy further improved through daily treatment with the antioxidant Cellfood. In the present study we examined the efficacy of long-term treatment, using both EDTA and Cellfood. Slow intravenous treatment with the chelating agent EDTA (2 g/10 mL diluted in 500 mL physiological saline administered in 2 h) (chelation test) removed Al, which was detected (using inductively coupled plasma mass spectrometry) in urine samples collected from patients over 12 h. Patients that revealed Al intoxication (expressed in μg per g creatinine) underwent EDTA chelation therapy once a week for ten weeks, then once every two weeks for a further six or twelve months. At the end of treatment (a total of 22 or 34 chelation therapies, respectively), associated with daily assumption of Cellfood, Al levels in the urine samples were analysed. In addition, the following blood parameters were determined: homocysteine, vitamin B12, and folate, as well as the oxidative status e.g. reactive oxygen species (ROS), total antioxidant capacity (TAC), oxidized LDL (oxLDL), and glutathione. Our results showed that Al intoxication reduced significantly following EDTA and Cellfood treatment, and clinical symptoms improved. After treatment, ROS, oxLDL, and homocysteine decreased significantly, whereas vitamin B12, folate and TAC improved significantly. In conclusion, our data show the efficacy of chelation therapy associated with Cellfood in subjects affected by Al intoxication who have developed ND.

1. Introduction

The risks for human health due to various physical and chemical aluminium (Al) forms (e.g. metallic Al, Al oxide, Al hydroxide and its soluble salts) have already been described in the literature [1,2]. Refinement of the provisional tolerable weekly Al intake, reduction of Al contamination in parenteral nutrition solutions, justification for routine addition of Al to vaccines, and harmonization of occupational exposure limits for Al substances have all been suggested. Aluminium is one of the most abundant elements on Earth used by man. It has no known beneficial role in any biological system. The increasing availability of products containing Al for human use has been related to both acute and chronic diseases [3,4]. Al has been shown to accumulate in many mammalian tissues, such as the brain, bones, liver and kidneys, where it exerts a toxic effect. The brain is considered to be the most vulnerable to the toxic manifestations of Al; indeed, Al has been proposed as a neurotoxin [5]. The negative impact of Al on the central nervous system (CNS) has been efficiently shown, emphasizing the potential link between Al and Alzheimer's disease (AD), together with amyotrophic lateral sclerosis (ALS) and autism spectrum disorders [6]. The toxic effect of Al on the rat hippocampus has been quantified, and apoptosis has been identified as the mechanism of Al-induced neuron death in this

brain zone [7]. Furthermore, evidence has been provided that neuronal metal ion imbalance-related oxidative stress contributes significantly to hippocampal injury caused by rat exposure to Al [8]. The role of oxidative stress and mitochondrial dysfunction in Al neurotoxicity has recently been highlighted [9]. The possible involvement of Al exposure (e.g. water, drugs, vaccines, cosmetics, industrial use) in human neurological diseases has also been reported [10].

We recently showed the relationship between Al intoxication and the development of neurodegenerative diseases (ND) [11]. Our patients affected by Al burden were treated successfully with the chelating agent EDTA (calcium disodium ethylene diamine tetraacetic acid) over a short period (ten chelation applications).

In the present study, we studied the effects of long-term EDTA treatment on patients affected by ND. Treatment was carried out once a week for ten weeks, then once every two weeks for a further six or twelve months (a total of 22 or 34 chelation therapies, respectively). We supported the patients with daily treatment using the antioxidant compound Cellfood, since its use has been shown to improve the blood oxidative status [12]. Accordingly, over time we monitored the decrease of the Al burden, the patients' clinical symptoms, and their blood oxidative and metabolic parameters.

2. Materials and methods

2.1. Patient recruitment

The 211 patients displaying Al intoxication were invited to undergo chelation therapy. Some were healthy subjects or controls, while others suffered from ND, such as multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD). Others were affected by non-neurodegenerative pathologies (non-ND, e.g. fibromyalgia). One healthy patient declined the proposal. All the others (n = 210 between 18 and 75 years old) were recruited for the present study. Each subject provided written informed consent.

2.2. Study design and evaluation of urine Al

The patients underwent a chelation test to assess Al intoxication in urine samples [11]. The patients were invited to collect the urine samples before and after intravenous treatment with the chelating agent EDTA (ethylenediamine tetraacetic acid, i.e. calcium disodium edetate, 2 g/10 mL diluted in 500 mL physiological saline, Farmax srl, Brescia, Italy). EDTA was slowly administered intravenously (the infusion lasted about 2 h). Urine collection following chelation lasted 12 h. The samples recovered from the collection were accurately enveloped in sterile vials and transported to the Laboratory of Toxicology (Doctor's Data Inc., St. Charles, IL, USA), where they were processed. Samples were acid-digested with certified metal-free acids; digestion took place in a closed-vessel microwave digestion system. Ultra-pure water was used for sample dilution. Testing was performed via inductively coupled plasma mass spectrometry (ICP-MS), using collision/reaction cell methods coupled with ion-molecule chemistry, a new and reliable method for interference reduction. Certified urine standards and in-house standards were used for quality control and to validate results. To avoid the potentially high margin of error that can result from fluid intake and sample volume, the results were reported in micrograms (μg) per g creatinine. Creatinine was measured by reverse-phase high-performance liquid chromatography, and was used to correct the total volume of urinary Al for differences in the glomerular filtration rates of individuals at the time of the spot sample [13]. Patients that showed Al intoxication following the chelation test underwent 10 weekly chelation therapies, and were then subjected to a further 24 applications once every two weeks for twelve months. After six and twelve months of chelation therapy (a total of 22 and 34 chelation applications, respectively) the patients underwent the same procedure used in the chelation test to show the presence of Al in the urine samples. The research programme, "Effects of Chelation Therapy with EDTA in Patients Affected by Pathologies Related to Exposition (Acute or Chronic) to Toxic Metals", was approved by the Ethics Committee of the University of Milan (Italy) (number 64/2014).

2.3. Evaluation of patient symptom improvement

The ability to work, the reduction of spasticity, relapse delay and fatigue disappearance were all considered, as previously described [11].

2.4. Cellfood treatment

Cellfood (Eurodream, La Spezia, Italy) is an antioxidant nutritional supplement containing 78 ionic/colloidal trace elements and minerals combined with 34 enzymes and 17 amino acids suspended in a solution of deuterium sulphate. It efficiently protects against oxidative damage in vitro [14]. Each of the 210 recruited patients received Cellfood for twelve months. A gradually increasing concentration of Cellfood was administered to subjects daily according to the following scheme: the first, second, and third day = 1 drop in mineral water three times a day, the fourth, fifth, and sixth day = 2 drops three times a day, the seventh and eighth day = 3 drops three times a day, that is, 1 drop more three times a day, and finally 20 drops altogether were given three times a day.

2.5. Evaluation of biochemical blood parameters

2.5.1. Sample collection

Biochemical parameters were measured in blood drawn from patients prior to performing programmed EDTA therapy, and following six and twelve months of EDTA treatment associated with Cellfood administration.

Peripheral blood samples were collected after overnight fasting into pre-evacuated and light-protected tubes, with no additives or with EDTA, to evaluate reactive oxygen species (ROS), total antioxidant capacity (TAC), oxidized LDL (oxLDL), glutathione, homocysteine (Hcy), folate (ery-Fol), and active vitamin B12.

Serum aliquots were used to measure ROS, TAC, oxLDL, active B12, and s-Fol (serum Fol) concentrations, while EDTA whole blood was used for glutathione and ery-Fol level determination. The remaining EDTA whole-blood sample was centrifuged within 30 min to obtain plasma for total Hcy determination. All the aliquots, except for the one used for blood counting, were immediately frozen and stored at −80 °C ready for assay.

2.5.2. Oxidative status

Serum ROS expressed as Carratelli Units (UCarr), oxLDL concentrations, and TAC were measured using a commercial enzyme-linked immunoabsorbent assay (ELISA, Mercodia, Uppsala, Sweden) on the EASIA reader (Medgenix Diagnostics, Fleurus, Belgium) and spectrophotometer commercial kits (dROMs test, Diacron International, Grosseto, Italy; OXY-adsorbent test, Diacron International) on F.R.E.E. analyser (Free Radical Elective Evaluator analyser, Diacron International), respectively.

Total and free glutathione concentrations were assessed with HPLC followed by fluorescent detection using a commercially available kit (Chromsystems Instruments & Chemicals, Munich, Germany). Total glutathione is the sum of oxidized (GSSG) and free (GSH) glutathione in the sample prior to reduction. Since chromatography can only determine the presence of GSH, the GSSG present in the sample was converted into GSH using a reduction reagent which reduced one GSSG molecule to two GSH molecules obtaining total glutathione. GSSG concentration was calculated by subtracting the GSH amount from the total glutathione. The GSH/GSSG ratio was also calculated and used as an oxidative stress marker.

2.5.3. Homocysteine metabolism

Plasma Hcy levels were measured using homocysteine liquid enzymatic assay (Sentinel Diagnostics, Milan, Italy) on Modular P analyser (Roche Diagnostics, Indianapolis, IN, USA). Serum active B12, s-Fol, and ery-Fol concentrations were determined using the relevant.

Abbott Microparticle Enzyme Immunoassay (MEIA) kits (Holotranscobalamin-Active-B12 and Architect Folate, Abbott Laboratories, Abbott Park, IL, USA) on Architect analyser (Abbott).

Lipid Panel. Serum total cholesterol (TC) concentrations were determined using the routine tests on Modular P analyser.

2.5.4. Statistical analysis

Data were analysed using analysis of variance (ANOVA), with the solution type as the main factor. Post hoc comparisons were made using Tukey's honestly significant difference test (HSD).

3. Results

3.1. Al intoxication and usefulness of chelation therapy

None of the examined patients revealed the presence of Al in urine samples without undergoing the EDTA chelation test (data not shown).

Patients that performed the chelation test with EDTA showed Al intoxication (211 out of 471 analysed). The remaining patients displayed intoxication with other toxic metals (e.g. Pb, Cd, As: data not shown). Among the patients with Al intoxication, 73 were healthy, 118 showed ND and 20 non-ND. Multiple sclerosis was seen to be present in 101 of the ND patients. All (except 1; n = 210) Al intoxicated patients underwent chelation therapy (once a week for 10 weeks, then twice a month for twelve months for a total of 34 chelation applications) together with daily Cellfood treatment. Table 1 shows the number of Al intoxicated patients (i.e. displaying Al levels in urine samples >35 μ/g creatinine) following chelation test, as well as after 22 and 34 chelation applications, respectively.

The number of patients displaying Al intoxication was reduced by chelation therapy, and reduction was more evident after 34 rather than after 22 applications.

Fig. 1 shows the levels of Al (expressed in μg/g creatinine) in urine samples examined after chelation test (i.e. before the beginning of chelation therapy, white colour) and after 22 (light colour) and 34 chelation applications (dark colour), respectively. Since ND patients were affected in particular by MS, we reported the Al values of both ND and MS patients. The Al values fell significantly following chelation therapy. Levels of Al in MS patients after 34 chelation applications were significantly lower than those obtained after 22 chelation applications. At a clinical level, patients treated with chelation therapy associated with daily Cellfood treatment showed reduced fatigue and disability.

Fig. 2 shows the biochemical parameters measured in patient blood.

The levels of active B12 and serum folate increased significantly after 22 and 34 chelation applications associated with Cellfood treatment compared to basal levels obtained before the beginning of therapy, both in controls and in ND patients. In parallel, homocysteine levels

Table 1
Number of patients affected by Al intoxication.
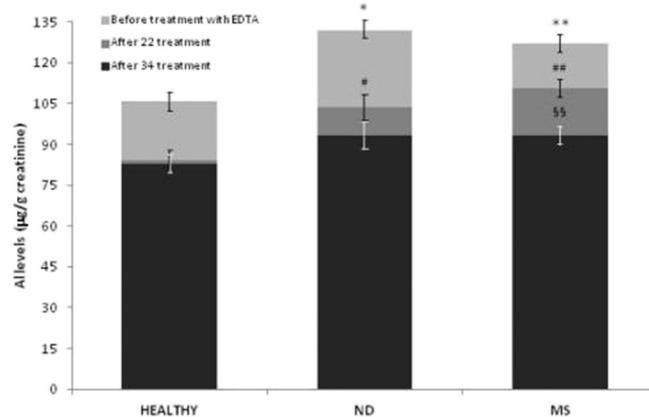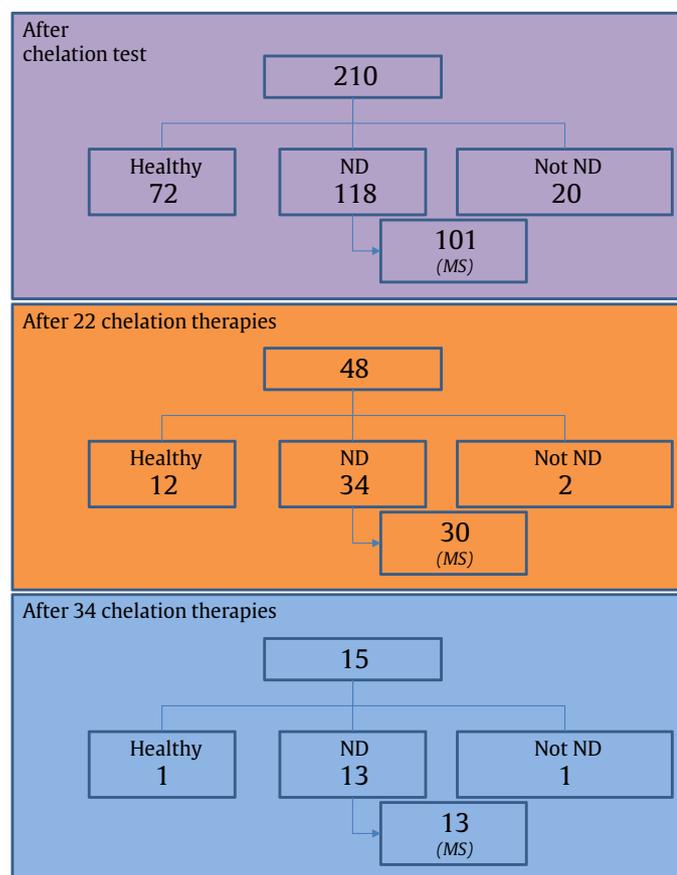ND = patients affected by neurodegenerative diseases.

Fig. 1. Aluminium (Al) levels evaluated in the urine samples of the studied subjects, following chelation test (light) and after 22 chelation applications with EDTA (middle light), or after 34 chelation applications with EDTA (dark), expressed as mean ± SEM of μg/g creatinine. The subjects studied were healthy patients, patients affected by neurodegenerative diseases (ND), and patients affected by multiple sclerosis (MS). The levels of Al in both ND and MS patients were significantly higher compared to those obtained in healthy subjects following chelation test (*P/**P < 0.05 vs. healthy). After 22 chelation applications with EDTA, Al levels in both ND and MS patients were significantly lower than those obtained following chelation test ($^{#}$P < 0.05 vs. *P and $^{##}$P < 0.05 vs. **P). After 34 chelation applications with EDTA, Al levels in MS patients were significantly lower than those obtained following 22 chelation applications (§§P < 0.05 vs. $^{##}$P).

decreased significantly. Basal ROS levels were more elevated, and basal TAC levels were lower in ND patients than in control patients. Long-term therapy was able to reduce ROS levels significantly, and increase TAC levels both in controls and in ND patients. Compared to basal values, TC and oxLDL levels decreased significantly in treated patients. Finally, basal GSH levels, which were lower in ND patients than in controls, improved significantly following chelation therapy associated with Cellfood treatment. The improvement was more evident in ND patients than in controls. On the whole, the therapy significantly improved oxidative status parameters.

4. Discussion

There is much evidence to support the role played by human exposure to Al in neurodegenerative diseases [5,6,12,15]. Recently, high levels of Al and manganese in the scalp hair samples of patients with neurological disorders (compared to controls) have been seen [16]. Elevated levels of Al in the brain have been related to the early onset of AD in patients exposed to Al in the workplace, suggesting a prominent role of the olfactory system and the lungs in the accumulation of this metal in the brain [17]. Moreover, chronic Al intake has been shown to cause AD, applying Sir Austin Bradford Hill's causality criteria [18]. Mitochondrial oxidative stress and dysfunction are known to be early pathological events that lead to neurodegeneration [19]. Since Al is involved in ROS generation [20], it might exert its effects by impairing mitochondrial function. The increased production of ROS inside the mitochondria might exacerbate oxidative damage to mitochondrial DNA and the disruption of oxidative phosphorylation, leading to cell damage and death. Many studies have indicated that the generation of oxidative stress, the release of calcium from intracellular stores, or the perturbation of mitochondrial function might represent important steps in the mechanisms underlying neuronal cell death induced by Al [21–23].

A recent review suggested the amelioration of neurotoxicity (i.e. oxidative stress and mitochondrial dysfunction) provoked by Al through the use of chelation therapy (deferoxamine), antioxidants, plant extracts/flavonoids, drugs and combination therapy [with N (2hydroxyethyl) ethylendiamine triacetic acid (HEDTA) and selenium] [9]. However, the role of metals in the pathophysiology of neurodegeneration cannot be limited to Al. In neurodegenerative disorders,
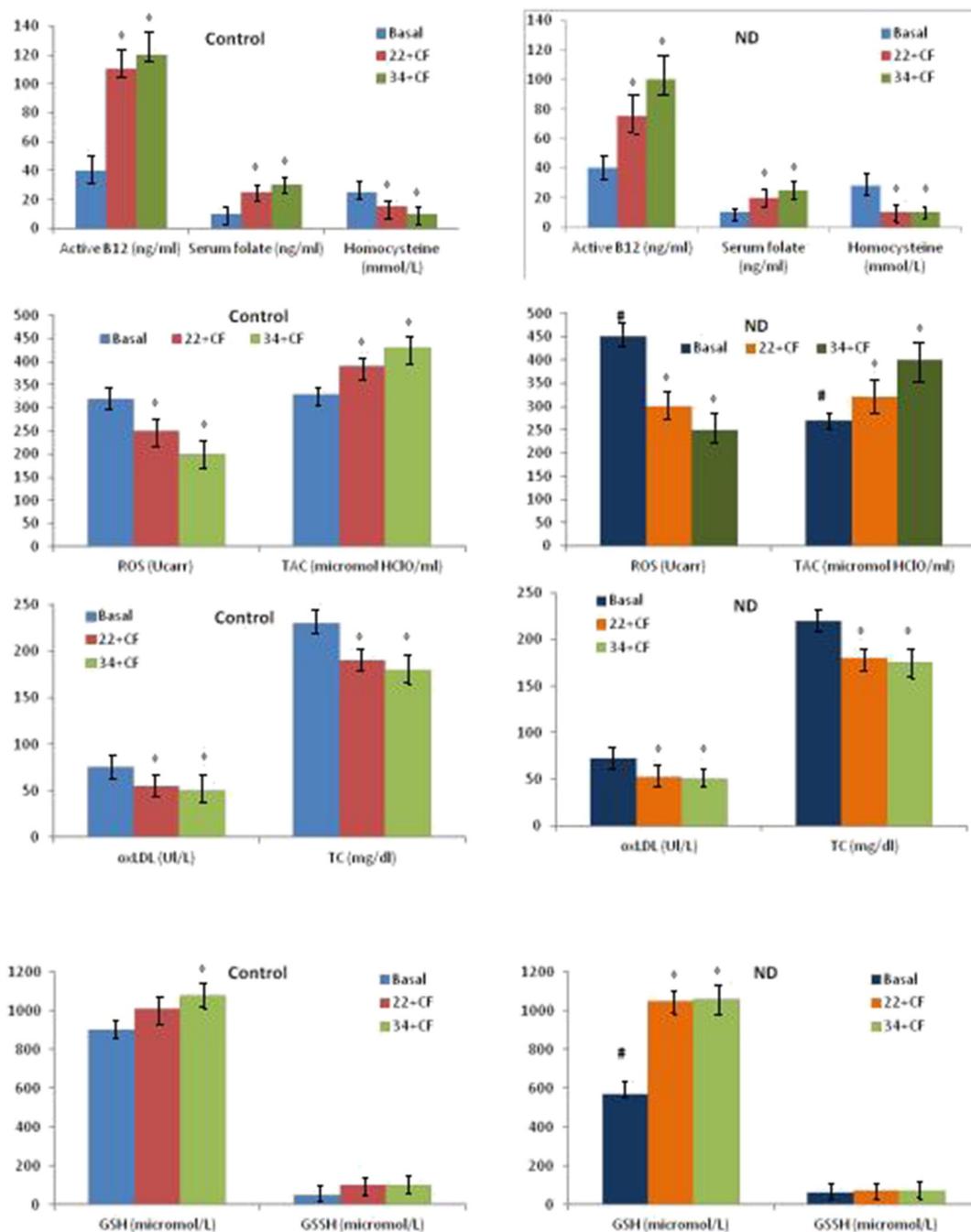
Fig. 2. Serum active Vit B12, folate, and homocysteine levels, serum reactive oxygen species (ROS) levels and total antioxidant capacity (TAC), serum oxidized LDL (oxLDL) and total cholesterol (TC), free glutathione (GSH) and oxidized glutathione (GSSH) levels and GSH/GSSH ratios measured in blood obtained from unaffected subjects (controls), or patients affected by neurodegenerative disease (ND). All values were determined before the beginning (basal values), and after 22 or 34 chelation applications associated with daily Cellfood administration. *P < 0.05 vs. the corresponding basal value; $^{\#}$P < 0.05 vs. control basal value.

oxidative stress, ROS activity, mitochondrial dysfunction, and energy failure might also be related to iron, copper and other toxic metals [24]. In this study, we relate successful EDTA chelation therapy (both in controls and in patients affected by ND) to the reduction of Al intoxication. However, the reduction of other toxic metals might also be responsible for neurological symptom improvement in these patients.

In a previous study, we showed the efficacy of short-term treatment with EDTA (ten chelation applications) in ND patients affected by Al intoxication [11]. Indeed, the neurological symptoms in treated patients improved in parallel with reduced Al levels in urine samples. In this study, we examined the effects of long-term EDTA treatment (22 and 34 chelation applications) in ND patients. We associated EDTA treatment to the daily administration of Cellfood, which has been

shown to improve oxidative and metabolic parameters in ND patients undergoing chelation therapy [12]. Our results show that Al levels, measured in the urine samples of patients affected by both MS (studied separately) and the unspecified studied neurodegenerative diseases, were significantly higher than those found in healthy patients. EDTA chelation therapy progressively and significantly reduced Al levels, and this reduction was more evident in ND patients than in controls.

The oxidative stress parameters improved significantly following combined treatment with chelation therapy and Cellfood. Indeed, blood ROS levels fell significantly, and TAC levels increased both in controls and in ND patients. This effect might be ascribed to EDTA, as it possesses antioxidant properties. In fact, chelation therapy has been shown to reduce oxidative DNA damage and lipid peroxidation without

any added vitamin C [25]. However, an important contribution was also due to Cellfood, which improved the mitochondrial respiratory metabolism of endothelial cells and inhibited hypoxia-induced ROS generation in vitro [14].

The significant increase of active B12, as well as serum folate, in our patients, associated with the significant reduction of homocysteine, oxLDL, and total cholesterol represent other important goals achieved by the combined treatment. Hyperhomocysteinemia is considered a risk factor for AD, and has also been associated with vitamin B12 deficiency [26,27]. Serum increase of oxLDL has been proposed as a putative good marker in MS patients because it correlates with the clinical activity (staging) of the disease [28]. GSH levels are also critical for cellular antioxidant defence. Some changes in the mitochondrial antioxidant systems have been observed in ND and acute brain disorders [29]. Although genetic disorders of GSH-related enzymes are rare,

GSH depletion is present in the pathogenesis of most major ND. Considering recent basic and clinical studies indicating that GSH depletion precedes neurodegeneration, neuronal GSH depletion might be a primary cause of ND [30]. The development of drugs to target neuronal GSH synthesis represents a promising therapeutic strategy for ND. Improved GSH levels in ND patients is another encouraging indication for the use of chelation therapy associated with Cellfood in Al intoxicated patients.

Acknowledgements

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