MISSED EVOLUTION OF DEMYELINIZING BRAIN LESIONS DURING SUPPLEMENTATION WITH NATURAL COMPOUNDS: A Case Report.

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system white matter, whose pathogenesis is incompletely understood. Plaques of demyelination are typically found in the periventricular and subcortical white matter. Evolution in space and time of new lesions from an initial Magnetic Resonance Imaging (MRI) report of demyelinating nature is not unusual, and it allows to formulate the diagnosis.

We describe the case of a woman in her late forties whom we examined, and after performing a cranial MRI showing multiple small lesions suggestive of inflammatory demyelinating nature. An oral daily integration of diet with natural compounds (i.e. galactose, Coenzyme Q10 and ferrous sulphate) was started. After 2 months of such supplementation numbness had disappeared, fatigue and central symptoms had sensibly ameliorated. A follow-up brain MRI was completed 6 months in welness, which showed that multifocal lesions were unchanged, even though enhanced by gadolinium. Data are discussed in consideration of the presence of an aerobic metabolism in myelin and of the implication of galactose as a preferential substrate for hexose 6-phosphate dehydrogenase recently reported in myelin. We discuss the evolution of the condition and missing the diagnosis of MS, and the possible neuroprotective role of the oral dietary integration. Today, the patient does not have any central symptom, is still under follow-up and treatment with the cited compounds and remains in a stationary condition.

Key Words: Multiple sclerosis, subcortical white matter, Magnetic Resonance Imaging, demyelinating disease, galactose

Abbreviations: CSF, Cerebrospinal Fluid; ETC electron transfer chain; Gal, galactose; MRI, Magnetic Resonance Imaging.

1. INTRODUCTION

Multiple sclerosis (MS) is the most common progressive demyelinating disease of the central nervous system (CNS) disabling the young [1]. It is a chronic inflammatory autoimmune disease characterized by demyelination and axonal degeneration of the CNS, whose nature and exact etiology is not fully established. The female-to-male ratio is 2:1 [2]. MS lesions are confined essentially to the white matter of the central nervous system (CNS), where demyelination occurs in discrete foci, termed plaques [1,3]. Axonal degeneration in acute phase is little, however loss of oligodendrocytes results in axonal degeneration at later stages. MS is believed to result from a cellular mediated autoimmune response against myelin components. Some reports have observed that MS is not a classical autoimmune disease in that it would not fulfil all of its required characteristics. Genetic and environmental factors have been implied in MS, suggestive of an overall multifactorial origin. A relationship among toxic action of metal pollutants has been proposed as a trigger for a primary myelin damage in MS, consistent with its predominant spread in industrialized countries [4]. Such geographic distribution has also suggested a viral agent or contributive agent to the disease, as yet not identified. MS diagnosis is based on the revised daily [5]. A brain Magnetic Resonance Imaging (MRI) is the most useful test for confirming the diagnosis of MS [6] [7]. MS lesions appear as areas of hyperintense signal on T_2-weighted and in T2-weighed fluid-attenuated inversion recovery (FLAIR) images, predominantly in the cerebral white matter. MRI imaging with gadolinium contrast following a first attack, can be helpful in providing evidence of lesions [7]. A second MRI scan may be useful three-to-six months after the initial lesion finding, and normally allows to identify new lesions and provide evidence of dissemination over time, getting to the diagnosis. Normally a progression in space and time is expected, even though there are some cases in which the lesions do not develop. In fact, MS often presents with a variability and diversity of symptoms and presentation, thereby including fatigue, and cognitive impairment.

D-galactose (Gal) is a sugar normally present in the body, pivotal for the early nutrition of mammals, as it represents 50% of lactose. Throughout human life, dietary Gal helps maintaining normal microbiota, increasing the number of Bifidobacteria in the gut [8]. Gal is widely distributed throughout the body, also in brain, which was shown to take up and metabolize Gal [9] across the GLUT3 hexose transporter, not insulin-dependent [10]. While it was shown that neurons generate ATP predominantly by oxidative phosphorylation (OXPHOS), which renders them vulnerable to energy failure, we have shown that myelin expresses functional electron transfer chain (ETC) and F_1F_o-ATP synthase and can aerobically produce ATP to support axonal mitochondria. This is consistent with Galactose being able to force cells to rely on aerobic respiration [11], by an unknown mechanism. Galactose administration was also shown to have a positive effect of MS patients [12].

Our patient developed in a short period of time (2 months) significant fatigue and a numbness on the right side of the face, all being features of MS. The MRI findings of our patient concluded that multiple areas of demyelination in the right semioval center and on the bilateral anterior frontal juxta-cortical white matter were present. The patient's condition was considered strongly suggestive of early MS and deserving strict subsequent follow-up. Galactose also when unphosphorylated can become substrate of hexose 6 phosphate dehydrogenase (H6PD, E.C. 1.1.1.47), a bi-functional microsomal enzyme that catalyzes the first two reactions of an endoluminal pentose phosphate pathway [13], generating reduced nicotinamide adenine dinucleotides (NADPH or NADH [14]) and Xilulose. Interestingly, a dysfunctional H6PD gene positively correlates with MS [15], suggesting that this enzyme plays an

important role for myelin sheath function. We have shown that Galactose is a better respiring substrate *in vitro* for myelin than glucose [16]. In fact myelin was reported to be able to conduct aerobic ATP production [17–20]. The use of Gal in clinical trials is limited to some cases of nephrotic syndrome or diabetic macular edema, and some interesting results are reported [21,22], also in an animal model of Alzheimler disease [23]. Here we considered the possibility to supplement the diet of our patient with an oral daily integration with galactose (3 g), Coenzyme Q10 (100 mg) and ferrous sulphate, (corresponding to 120 mg elemental iron), initiated immediately. After 2 months, the central symptoms had disappeared. After 6 months, a second MRI was done, which showed a non-evolving situation and the Patient missed the diagnosis of MS.

2. CASE REPORT

2.1 Clinical Presentation.

The Patient is a 48 year-old white female. She came to our attention for her recent neurologic complaints. She referred that in the last 2 months at the time she had numbness on her right side of the face, slightly impaired short-term memory, and severe fatigue. She also referred newly diagnosed anemia. Personal history was significant for Hashimoto thyroiditis and acute leukemia as a child (followed by complete recovery) as well as for gluten sensitivity later in life. Celiac disease was excluded, as the Patient does not express HLA DQ2 nor DQ8. The patient was evaluated by neurology after the episode, and had a normal physical and neurological exam as for cranial nerves motor examination, deep tendon reflexes, and sensory exam. There were no loss of balance, or blurred vision. Funduscopic examination was reported normal.

Peripheral blood tests showed negative anti-gliadin, transglutaminase Antibodies (Ab) and EMA; anemia (Hb value was 8.8 g/dL). Erythrocyte sedimentation rate, biochemistry, liver function, thyroid-stimulating hormone level, C-reactive protein, were negative. A panel of IgG against CMV, EBV, HSV-1, HSV -2 and Zoster viruses was conducted. CMV resulted negative, while our patient was found to carry a reactivation of EBV and Zoster. Her immunologic state was also examined with a lymphocyte comprehensive panel, that evidenced slight reduction in memory (Senescent $CD8^+$) and Suppressor cytotoxic ($CD8^+$) lymphocytes, while the regulatory T lymphocytes were at the lowest levels of the normal range, consistently with the history of Hashimoto thyroiditis.

The patient brought an MRI scan performed at that time, according to axial, sagittal and coronal scans by the SE/EP, FSE technique. Figure 1 shows 9 scans corresponding to T2-weighted FLAIR signal lesions. MRI showed multiple small areas of altered size and increased signal intensity in the right semioval center and on the bilateral anterior frontal juxta-cortical white matter, suggestive of inflammatory demyelinating nature deserving clinical correlation and evolutionary controls. Based on the brain MRI findings, visually or somatosensory evoked potentials or Cerebrospinal Fluid (CSF) analysis were not recommended. However, an oral integration of diet with nautral compounds (i.e. D-galactose, started integration with D-galactose (3 g/die), Coenzyme Q10 (CoQ, 100 mg/die) and also ferrous sulphate, (one 300 mg tablet containing 60 mg elemental iron) two times daily. After 2 months of such dietary integration the numbness had disappeared and has never come again, and attention and fatigue and central symptoms had sensibly ameliorated.

A follow-up brain MRI was completed 6 months by VTD1W 3D MR technique, in complete wellness at the time, before and after contrast medium (Magnevis, 15 ml, e.v.) in the three planes of space. Figure 2 shows unchanged multifocal contrast-enhanced T1-weighted signal alterations of brain hemispheric white matter, with respect to the first MR scan, even though these were enhanced by

gadolinium. The second MRI could not confirm a diagnosis, of MS. Consequently, as the previous diagnostic hypotheses remained valid, the recommendation of the neurologist and radiologist was to continue follow-up MRI in 12 months. Now the patient is in complete well-being and under follow-up.

3. DISCUSSION

We report herein a patient presenting with typical MS features (paresthesia, and fatigue). The patient also complained of a cognitive disability resulting in slight functional impairment at work, which has been described in MS patients. Such central symptomatology did raise the suspicion of MS. Consequently, the neurologist suggested performing an MRI.

MRI is widely utilized as the most important paraclinical tool for diagnosing MS and therapeutic monitoring, playing a dominant role in ruling in or out a diagnosis of MS. MRI offers the most sensitive way to detect MS lesion [6]. MS is heterogeneous in terms of clinical forms. MS plaques are found throughout the brain, especially in the periventricular and deep white matter, and subcortical region. The early acute stage is believed to correspond to myelin breakdown by perivascular inflammation and disruption of the blood-brain barrier (BBB), leading to gadolinium enhancement detectable by MRI [24]. This was the case also for our patient (Fig 2). The focal demyelinating lesions located along the corpus callosum were depicted by sagittal T2-weighted FLAIR imaging (Fig 2). MRI T2-weighted FLAIR lesions were typically hyperintense (Figs 1 and 2). T1-weighted imaging (data not shown) of lesions was isointense to the normal white matter.

MS is a challenging disease, especially in diagnosis. In some cases, after the first episode, no new lesions occur, and these never develop to clinically definite MS. However, many patients who suffer an isolated monosymptomatic episode of demyelination, will ultimately develop a second inflammatory event. This is true especially if the first MRI reveals white matter changes characteristic of MS, like in our case, and in case the patient shows an auto-immune trait. Sometimes patients are classified as suffering from MS tout-court after the first suggestive lesion. Our patient was instead advised to perform a second MRI after 6 months. Follow-up scans are recommended, as in the present case, to reach a diagnosis (objective evidence of two or more neurologic signs localized to the CNS, in different parts of the brain or spinal cord in two separate episodes at least three months apart) [5]. In the case of our Patient, the condition is stationary as shown by the 9 contrast-enhanced T1-weighted scans. In patients with MS, perfusion-weighted imaging highlighted a diffuse brain hypoperfusion and hypoxia [25], both causative of cognitive dysfunction and fatigue, and inducing mitochondrial energetic failure and oxidative stress. Hyperintense T2-weighted FLAIR MRI lesions in patients with MS are tipically situated in lower perfused white matter areas [25]. A significant reduction in the expression of mitochondrial electron transport chain proteins was also reported [26]. However, besides axonal mitochondria, myelin sheath was also reported to play a role in supplying the axoplasm with ATP, aerobically produced in its spires with a prominent role for connexons [27–29]. In fact, the mitochondrial F_1F_o-ATP synthase, and the respiratory complexes are expressed in myelin [30–35]. An extra-mitochondrial oxidative phosphorylation in myelin needs heme synthesis, to supply cytochromes, consistent with the iron content of myelin sheath as measured by MRI [36]. Myelin-forming oligodendrocytes stain for iron, and decreased availability of iron in the diet is associated to hypomyelination [37]. This can be correlated , in our patient, with anemia.

Iron deficiency causes severe impairment of myelination, during development [38]. Iron plays a role in MS, when for example an elevated ferritin content in serum of MS patients was found [39]. An iron deficiency would imbalance

the electron transfer chain (ETC) functioning both in axonal mitochondria and in the sheath, impairing oxygen absorption and causing oxidative damage. Restoring CBF emerges as a new therapeutic target in MS, but this also applies to curing anemia. Following this line of thought, the correction of anemia with an oral supplementation of Fe^{2+} salts may have been beneficial in our patient, in fact anemia and hypoxia can conceivably be considered a risk factor for MS, especially in a person bearing a unbalanced immune response, as our patient is.

As for the oral integration with Gal, a monosaccharide normally present in the body, its role in neuroprotection may have been long neglected. First of all, Gal is the very first nutrient for mammals, in a 1:1 ratio with glucose and in the adult can enter the blood–brain barrier in a manner independent of insulin secretion [10]. Clinical trials on treatment of MS patients with Gal are presently lacking. However, clinical trials were conducted on the use of Gal for patients with Diabetic Macular Edema, Focal Segmental Glomerulosclerosis and Steroid Resistant Nephrotic Syndrome. In particular, results are known of a Phase II clinical trial (NCT00814255) designed to assess the efficacy of adalimumab and galactose, for patients with resistant focal segmental glomerulosclerosis (FSGS) unresponsive to corticosteroids and other immunosuppressants. Interestingly, some subjects in the Gal arm had a reduction in proteinuria without a decline in eGFR, while adalimumab did not achieve the primary target [22]. A case of focal segmental glomerulosclerosis, in which the immunosuppressive therapy had failed to induce remission, was treated with oral galactose for one month. The effect of oral Gal administration at a dose of 0.2 g/kg twice a day was a partial remission of resistant nephrotic syndrome with a decrease of proteinuria by 50%, and normalized the plasma albumin and cholesterol [21]. The underlying mechanism has not yet been clarified. The association of Gal and antioxidants, such as Coenzyme Q10A may represent a basic neuroprotection supplementation. In fact, Gal, if adequately supported by antioxidants, may represent a "rescue " substrate for the nervous tissue and in particular for the myelin sheath. Glucose is too costly to be used *de novo*, as it requires prior phosphorylation to be utilized as a respiring substrate in conditions of chemical energy shortage. This is the case of demyelination, and also of the newborn, that is in fact fed with milk containing 7 grams/kg of lactose, composed by 50% glucose and 50% Gal. Gal becomes part of cerebrosides, but may also play a catabolic role: our preliminary results show that Gal is a better respiring substrate for myelin *in vitro* than glucose [16], with the possible participation of H6PD [13,40].

Gal simultaneously administered i.v. and orally to MS patients was able to ameliorate the most invalidating symptoms [12]. Although dated, the cited report is interesting, as it underscores the role for Gal in the CNS. More recently, a beneficial effect of oral Gal was reported in preventing the development of the cognitive deficits in the streptozotocin-induced rat model of sporadic Alzheimler's disease [23]. Gal would be a respiring substrate for extra-mitochondrial ATP synthesis and oxygen consumption in myelin, which expresses H6PD, a microsomal enzyme displaying a particularly favorable K_M for galactose, that has recently been considered a risk gene for MS [15]. In the context of myelin sheath, expressing the ETC [17–20], Gal, also unphosphorylated, would be the substrate of H6PD, generating reduced nicotinamide adenine dinucleotides (NADPH or NADH [14]) for Complex I of ETC and Xilulose, an activator of glycolysis [41]. This would be consistent with the reported ability of Gal to force cells to rely on aerobic respiration [11]: Gal-grown HepG2 cells are more reliant on OXPHOS rather than glycolysis, and double Oxygen consumption [42]. H6PD is membrane-bound and has high affinity for several phosphorylated and non-phosphorylated hexoses, among which Gal, Gal 6-phosphate, 2-deoxyglucose, 2-deoxyglucose 6-phosphate, glucosamine 6-

phosphate, and glucose 6-phosphate [14,43]. Its physiological role is classically considered exclusively related to steroid metabolism [40], but following the hypothesis that the mitochondrial OXPHOS machinery is embedded in myelin, as suggested by many proteomic reports [44] and considering that also H6PD is expressed in myelin [16], it would be functionally associated with Complex I of respiration, producing NADH for the ETC, as we have discussed [45]. Recently H6PD has been indicated as a risk factor for MS [15], suggesting that this enzyme plays an important role for myelin sheath function. Future biochemical experiments may prove this hypothesis. This was the rationale for the integration with Gal. On the other hand, the supplementation with CoQ10 appears correct for the function of this coenzyme in funneling electrons through the ETC. Notably, iron supplementation may also have exerted a positive action on the functionality of the extra-mitochondrial ETC expressed in patient's myelin, which needs cytochromes [17–20]. In fact, a heme synthesis has been reported to occur in myelin [46].

In conclusion, disease activity in MS is strongly linked to the formation of new lesions, therefore, for the repeated analyses of new lesion formation, MRI provides relevant data [47]. Here, it is important to acknowledge that, with a dietary integration, the patient did not develop a worsening of her condition. In this respect, the integration with galactose/Coenzyme Q10, may be an approach intended to support the myelin bioenergetics, to exert a neuroprotective effect, reducing further injury to the white matter. Notably, supplementation with Gal and antioxidants is recommendable, as in the present case, especially in the very early stages of the suspect or new diagnosis of MS, from the considerations cited.

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6. FIGURES

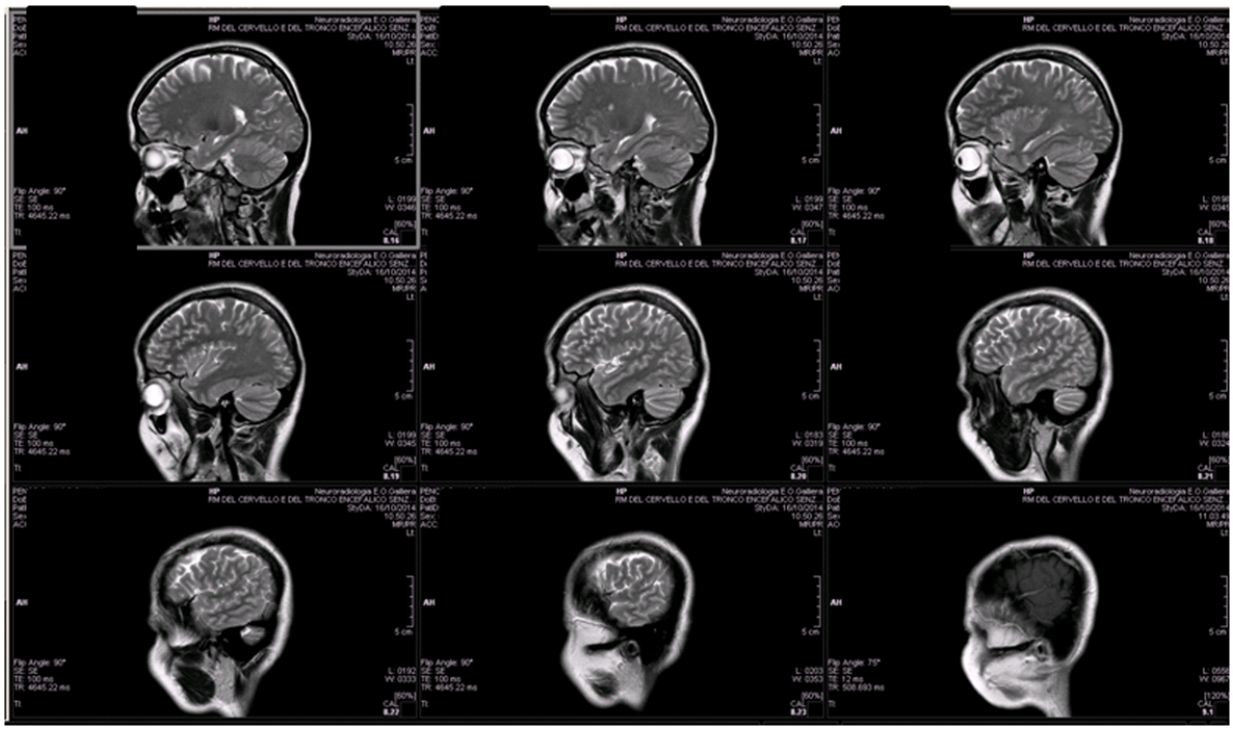

Figure 1. Cranial magnetic resonance imaging at the time of the consultation. Sagittal MRI scan T2-FLAIR sequences of the brain of the Patient showing multiple demyelinating lesions that are hyperintense relative to the normal appearing brain tissue. The 9 sagittal FLAIR sequential scans shows the multiple areas of increased signal intensity in the left semioval center and on the bilateral anterior frontal juxta-cortical white matter, of a demyelinating nature. FLAIR, fluid attenuation inversion recovery.

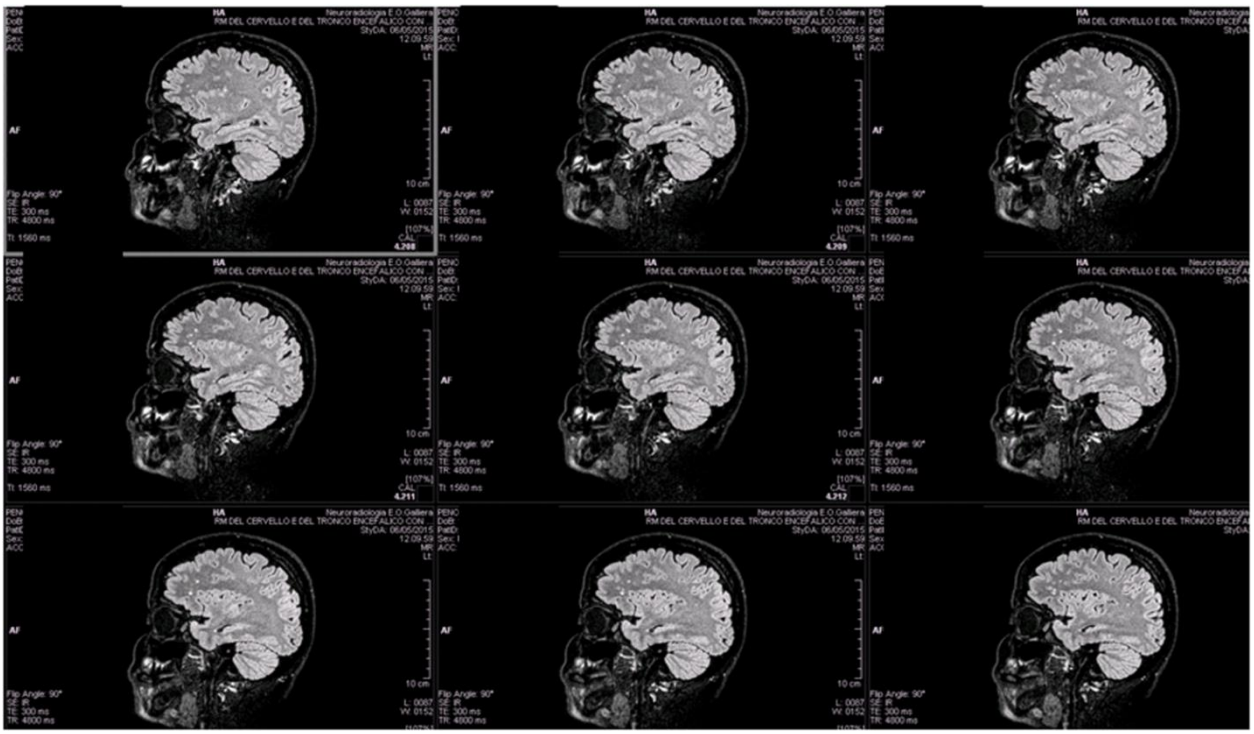

Figure 2. Cranial magnetic resonance imaging 6 months later. Sagittal contrast-enhanced T1-weighted brain magnetic resonance imaging scans obtained serially at 6 month interval in our patient. Unchanged multifocal contrast-enhanced T1-weighted signal alterations of brain hemispheric in the left frontal white matter, with respect to the first MR scan, even though these was enhanced by gadolinium. No formation of new plaques was seen.

