Personalized Prevention in Mercury-Induced Amyotrophic Lateral Sclerosis: A Case Report

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Featured Application: Knowing the effects observed in a worker exposed to mercury can improve the prevention of the risks associated with this toxic metal.

Abstract: Chronic exposure to low levels of mercury is involved in the development of motor neuron diseases (MND). Genetic alterations may have a crucial role in the onset and progression. We presented a case of a TANK-binding kinase 1 (TBK1)-mutated 54-year-old male worker who developed a MND due to chronic mercury exposure at work. He was employed in a chlor-alkali plant in Central Italy. After two years of employment he had acute mercury intoxication with suggestive neurological symptoms and a high urinary level of the metal. Through years, many episodes of intoxication occurred, but he continued to perform the same job and be exposed to mercury. After yet another episode of intoxication in 2013, he showed fasciculations of the upper limbs and trunk, and electromyographic activity patterns were consistent with amyotrophic lateral sclerosis (ALS). In 2016, a genetic test revealed a mutation of TBK1, an ALS-related gene. This case highlights the important role of genetics in personalized occupational medicine. Occupational physicians should use genetic tests to identify conditions of individual susceptibility in workers with documented frequent episodes of mercury intoxication recorded during health surveillance programs to customize prevention measures in the workplace and act before damage appears.

Keywords: mercury toxicity; motor neuron disease; occupational neurotoxicity; chlor-alkali plant; factory worker; personalized medicine; genetic susceptibility; genetic test; elemental mercury

1. Introduction

Mercury (Hg) is a naturally occurring toxic heavy metal [1,2] and a global pollutant [3]. Occupational exposure to elemental inorganic mercury may cause neurological and other adverse effects in exposed workers [4–6]. Even if its neurotoxic effects have been known for more than 300 years [7], occupational exposure may still be relevant in certain activities such as mining and metal working [8–10], production and waste of fluorescent lamps [11,12] and incinerators [13,14], recycling [15,16], concrete processing [17], battery production [18], thermometer and precision instrument manufacturing [19], dental care [20], and other industrial activities. Chlor-alkali plants

have been extensively studied over the past 60 years for being at the origin of the ecological disaster of Minamata [21] and for neurological pathologies in exposed workers [22–31].

Trends in production and sales of Hg are rapidly increasing according to high global Hg demands (Global Chemicals Outlook II, 2019) [32]; consequently, the number of exposed workers is increasing. Occupational risk prevention is based on the adoption of environmental prevention measures, good work practices [33], and biological monitoring of workers. Mercury in urine (U-Hg) is commonly used to indicate exposure in occupational cohorts [34]. The reference values for U-Hg have been progressively reduced over years; for example, the American Conference of Governmental Industrial Hygienists (ACGIH®) has reduced the Biological Exposure Index (BEI®) for U-Hg from 35 (2007) to 20 μg/g (2017) [35].

A systematic review of neurotoxicity studies in Hg-exposed workers confirmed dose-relatedness of objective neurological effects, including tremor, impaired coordination, abnormal reflexes, and reduced performance on neurobehavioral tests of manual dexterity and motor speed [36]. The metal mainly affects the central nervous system and is expressed in typical motor neuron diseases (MNDs). It has been long known that MNDs have a polyfactorial pathogenesis, and environmental influence has been associated with sporadic forms of amyotrophic lateral sclerosis (ALS), which is the most common MND in adults [37]. Moreover, huge differences in the onset and severity of neurological symptoms in individuals with a comparable level of exposure to Hg have been reported. This points up the role of the underlying genetic factors that may modify Hg uptake, biotransformation, distribution, and elimination, and, as a result, the effective dose, i.e., the dose that produces a biological response [38]. Furthermore, through the years, several epigenetic and genetic characteristics have been associated with Hg neurotoxicity [39].

In this short paper, we reported the case of a worker exposed to Hg in a chlor-alkali plant, who presented several episodes of work-related neurotoxicity and, finally, developed ALS. Genetic tests showed that he had a TANK-binding kinase 1 (TBK1) mutation.

2. Case Presentation

A 54-year-old male worker has been working in a large chlor-alkali plant in Central Italy since 1986 as a small industrial plant maintenance contractor. He mainly performed maintenance activities in Hg cells, where Hg cathodes were working in brine (saltwater—mainly sodium chlorine or, to a lesser extent, potassium chloride), or was assigned to piping and filter cleaning. He was exposed to inorganic Hg, as well as other chemical (chloromethane, acids and bases, welding fumes) and physical (noise, heavy load handling) agents.

The medical history was not contributory, but the familiar history was noticeable for neurological disorders in first degree relatives (i.e., his mother was affected by Parkinson's disease and deceased from a stroke, two brothers were affected by myogenic torticollis). He had middle-lower education and was not a smoker. The patient followed the Mediterranean diet, consuming fish on average 3–5 times per month.

He was subjected to periodic medical examinations supplemented by biological monitoring of urinary Hg tests (U-Hg) through occupational health surveillance programs.

In 1988, after working on cleaning mercury cells, he manifested tremor, vertigo, marked weakness, ataxia, and fever. U-Hg level of 127 μg/g creatinine, significantly higher than the biological exposure limit BEI® (20 μg/g creatinine), was observed. He was temporarily removed from the job for two weeks, but no occupational injury was reported to the competent authorities. In the following years, a certain number of episodes of malaise, dizziness or confusion, agitation, and insomnia, associated with U-Hg values between 60 and 80 μg/g creatinine, occurred. None of these recurrent episodes of subacute intoxication were reported to the competent Italian authorities. The occupational physician responsible for medical surveillance of workers declared him "fit for work". As a result, he was employed with the same working tasks for over 20 years. In 2008, the same occupational physician prescribed that

he could not be exposed to respiratory irritants and chemical risk. Despite this restriction, however, he continued his previous activities.

In October 2012, at the end of the work shift, he reported unpleasant metallic taste, nausea, dry mouth, difficulty in swallowing, and voice lowering. In the following working days, other symptoms appeared, such as marked weakness, muscle cramps, intentional tremors hindering work performance (e.g., welding of metal staples). In August 2013, fasciculations of the upper limbs and trunk were also evident. Electromyographic examination showed denervation potentials in the upper limb muscles (deltoid muscle bilaterally and the right vastus lateralis) and abnormal spontaneous activity in the lower limb muscles (plantar interosseous and anterior tibial muscle, bilaterally).

In December 2013, he came to our attention. Neurological examination showed high-frequency postural tremor (right side > left side) occurring in standing position, with dysarthria and tongue fasciculations, weakness and atrophy of the small hand muscles, fasciculation in all four limbs, with no signs of pyramidal tract involvement. Hematochemical parameters were normal. Brain MRI scan documented no signs of brain impairment. In March 2014, lung function tests showed a restrictive deficit, supposed to be a consequence of limited diaphragm excursion due to neurological impairment.

Genetic screening for Kennedy disease showed a negative result in the target gene (Androgen Receptor, AR). In consideration of the history of recurrent episodes of Hg intoxication, we reported the suspicion of occupational mercury poisoning to the National Italian Occupational Insurance Authority (INAIL), which refused the claim. The patient continued to be treated by the Neurology Department of our university hospital, which diagnosed amyotrophic lateral sclerosis. Over the following years, he presented progressive weakness involving proximal upper limbs and respiratory muscles. Due to respiratory failure, he received mechanical ventilation through a tracheostomy, 36 months after the onset of the symptoms. In 2016, further analysis of major ALS genes using the technique of next generation sequencing, revealed deletion c.1852_1854delGAA: p.E618del in the TBK1 gene.

In subsequent years, lung function parameters were getting worse and nocturnal apnea appeared. Meanwhile, he sued the INAIL Institute, thus obtaining that the disease was recognized and compensated.

3. Discussion

In this case report, we showed a worker who was chronically exposed to Hg in a chlor-alkali plant and developed ALS after recurrent episodes of neurotoxicity. Interestingly, genetic testing carried out in this worker showed a TBK1 mutation (deletion in c.1852_1854delGAA: p.E618del). ALS is the most common neurodegenerative disease affecting motor neurons. The diagnosis of ALS is based on two different criteria: the revised El Escorial criteria (rEEC) [40] and the Awaji criteria [41], that may have overall superiority [42], though they are not always more sensitive than rEEC [43]. The criteria have high inter-rater variation [44]. Although up to 10% of ALS is heritable (familial ALS) and, in most cases, caused by genetic mutations, the majority of the cases of this fatal neurodegenerative disease occurs in individuals with no prior family history (sporadic ALS), and is of unknown etiology. As it is a complex and heterogeneous disease, polyfactorial interaction between genetic characteristics, metabolic, and environmental factors might be at the basis of sporadic ALS cases [45]. Regarding the genetic factors, through the years, several genetic mutations which interfere with the onset and progression of the disease, have been associated with ALS, being either causative or disease-modifying. Mutations can have different cellular sites of attack: coding of proteins implicated in the antioxidative response system (e.g., SOD1), RNA stability, function and metabolism, nucleocytoplasmic trafficking (e.g., ANG, TARDBP, FUS, HNRNPA1, MATR3), cytoskeletal dynamics (e.g., DCTN1, PFN1, TUBA4A), the ubiquitin-proteasome system (e.g., VCP), mitochondrial functions (e.g., CHCHD10), and autophagic pathways (e.g., C9ORF72, OPTN, UBQLN2, SQSTM1, VCP, CCNF, TBK1) [46]. In particular, mutations in TANK-binding kinase 1 (TBK1) have been associated with the sporadic form of ALS and other neurological diseases such as frontotemporal dementia [47]. The frequency of TBK1 variants is 0.5–1.6% in pure ALS patients [48], and 3.5–4.5% in ALS cases with frontotemporal dementia [49]. The protein

encoded by TBK1 is a serine/threonine protein kinase, which has an important role in the induction of autophagy and innate immune response pathways [50]. Moreover, TBK1 variants are associated with reduction of the corresponding protein level [51], leading to accumulation of protein aggregates and defective mitochondria in motor neurons, which may contribute to ALS pathology by disrupting axonal transport [50].

In addition to and, most probably, in association with the genetic background, a proportion of ALS cases are likely to be attributable to occupational exposure. High-dose occupational exposure of workers in the 1950s and 1980s induced several cases of neurological impairment similar to ALS, which regressed after cessation of exposure [52–55]. Nowadays, low-dose occupational exposure of sensitive individuals remains an occupational health concern worldwide.

Genetic polymorphisms and epigenetic changes have been correlated to different health effects of Hg in exposed workers [56]. For example, it is known that Hg can induce direct and indirect DNA post-translational modifications (PTMs) such as phosphorylation, ubiquitination, acetylation, nitrosylation, and S-mercuration on targeted proteins implicated in many cellular pathways [57,58]. Specific polymorphisms of glutathione-related genes (e.g., glutamate-cysteine ligase regulatory subunit, glutamate-cysteine ligase), Hg transporter proteins (e.g., MTs, ABCs), cytochrome p450 3A, ε4 APOE, and Brain Derived Neurotrophic Factor (BDNF) have been associated with the magnitude of toxic Hg effects in biological tissues [39,58–61]. The induction of oxidative stress, which can activate the process of mitochondrial autophagy, is the initial step of Hg neurotoxicity [62]. Autophagy represents an important defense mechanism in cells that allows to selectively eliminate organelles and/or dysfunctional proteins, subsequently reusing their molecular components. Exposure to toxic metals (Hg included) can induce a series of cellular metabolic and energetic alterations, including faulty autophagy [62,63]. Moreover, up-to-date evidence highlights the existence of mitoautophagy, a self-destructive mitochondrial dysfunction which occurs at early stages of ALS [64]. Mitochondrial autophagy can be hypothesized as a common link for both ALS and Hg intoxication. In fact, Hg ions have been implicated in faulty mitochondrial autophagy even with continuous low-dose exposures [63]. Studies associating mercury and ALS have been conducted mainly with methylmercury, which is the most toxic form of the metal. In experimental conditions, methylmercury (MeHg) caused LDH release, caspase activation, cell-cycle alteration, and ROS generation, in accordance with a decrease in cell viability [65]. The neurotoxicity of Hg (particularly MeHg) is associated with three main mechanisms: (1) increase of intracellular Ca^{2+} levels; (2) induction of oxidative stress by overproduction of ROS or by reduced oxidative defense capacity; (3) interactions with sulfhydryl groups with the formation of a thiol-containing complex [66]. Intracellular calcium levels also appear to be linked to autophagic processes within cells [67]. Autophagic processes and the formation of autophagosomes increase in cells exposed to Cys-S-Hg-S-Cys [68]. $HgCl_2$ treatments decreased mitochondrial functionality, suggesting that nonessential metals compromise mitochondrial homeostasis and activity [69]. Moreover, the binding of MeHg to GSH is known to decrease the availability of this antioxidant, exposing cells to free-radical-mediated damage [70].

Since ALS has a multifactorial genesis, in the reported case, both genetic predisposition (pathogenic TBK1 variant) and occupational Hg exposure concurred in the development of the disease. A conceivable hypothesis suggests that acute/subacute subclinical reduction in the motor neuron pool may occur with the initial phases of occupational exposure to Hg, but the defect remains unrecognized until that pool becomes further depleted through the normal aging process [71] or the additive effect of several successive occupational exposures.

This case proved the key role of health surveillance in the workplace. The great variability in the individual responses of workers requires strong attention by the occupational physician, who must promptly identify the first symptoms of an excessive response. In the reported case, the observation of intermittent subacute flares of Hg intoxication, firmly assessed through high Hg levels in urine, should have been considered a red flag that must induce deeper analysis of the worker's genetic pattern long before the onset of the neurological disorder.

The intervention of the occupational physician to prevent occupational mercury intoxication should be directed primarily at modifying the environment, rather than assessing the worker's adaptation to the job. However, a small maintenance contractor has little chance of influencing the chlor-alkali plant safety strategy. In these cases, the decision to allow exposure to risk, declaring the worker "fit for the job", or to declare him unfit, exposing him to dismissal, is very difficult. Even if the causes of ALS are multifactorial and a single gene mutation might not be sufficient to cause ALS, personalized occupational health practices and searching for genetic biomarkers of susceptibility in selected cases with documented frequent episodes of mercury intoxication recorded during health surveillance programs could be an aid in the doctor's decision, allowing to customize prevention measures in the workplace and act before serious and irreversible consequences of occupational exposure appear. The use of genetic tests must be reserved for specifically motivated cases and should not be considered a universal screening measure. Ethical considerations [72] and the Italian Constitution [73] do not allow occupational physicians to perform genetic screening tests in the pre-assumptive phase. Moreover, the search for all the genes that could be associated with increased mercury toxicity is expensive and complicated and, therefore, cannot be applied to everyone, but must be reserved for cases with reasoned suspicion. Conversely, inappropriate genetic testing could easily become a basis for discrimination. Laws were produced against this danger, such as the Genetic Information Non-discrimination Act (GINA) in the USA, and similar laws in other countries [74], even if uniform protection of workers from genetic discrimination is still far from being reached [75]. The aim of the occupational health practice remains "to take into account workers' state of health to sustain and improve their working capacity and ability, and contribute to the establishment and maintenance of a safe and healthy working environment for all", as stated in the International Code of Ethics for occupational health professionals [72]. We must orient genetic research to this goal.

The main limitation of this study is the report of a single case. The low incidence of ALS (1.75/100,000 person/years) [76] makes it difficult to collect cases in the workplace. The main strength of the study is the collection of occupational history of over 20 years.

4. Conclusions

In conclusion, our work gave further data in favor of the potential role of inorganic Hg in ALS genesis. Furthermore, it provided significant insights on the magnitude of investigations and monitoring activity at the workplace for early diagnosis of occupational diseases. A better understanding of the molecular mechanism of Hg poisoning and its potential interaction with the genetic background may open new horizons for reducing occupational risk.

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