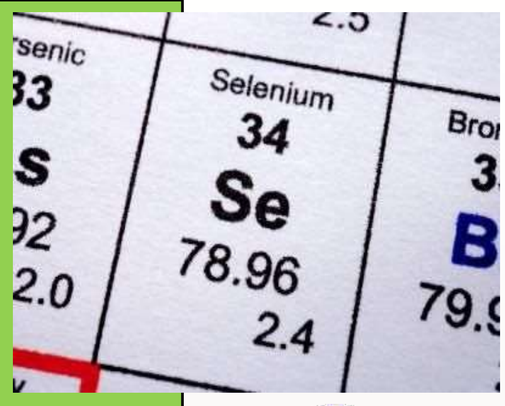
Selenium species and heavy metals in cerebrospinal fluid and risk of amyotrophic lateral sclerosis: a hospital-based case-control study



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Background and aims

Amyotrophic lateral sclerosis (ALS) is one of the diseases which has been ascribed to overexposure to heavy metals and the metalloid selenium, but few data are still available on this issue, including the involvement of a specifies selenium species, which have very different biological activities. Using an advanced analytical methodology, we designed a case-control study to test the association between selenium species and heavy metals with ALS risk, using as biomarker of exposure their concentrations in cerebrospinal fluid.

Methods

In a case-control study, we determined the Se compounds and the levels of Cd, Hg and Pb in cerebrospinal fluid samples of 38 ALS patients, recruited in the ALS Center of the Neurological Clinic of Modena University Hospital from May 1997 to April 2011, and of 38 reference neurological patients (16 men and 22 women; mean age 38 years) with signs or symptoms of suspected, but unconfirmed: liquoral hypertension or hypotension (3), primary headache (10), paresthesias with negative instrumental evidence (5), isolated idiopathic cranial nerve abnormalities (6) and other. The final diagnoses have been: migraine/cephalea (n=10), parhesthesias (7), cranic nerves abnormalities (6), mild mental confusion (3) and other disorders outside major neurological disease groups. The controls were matched 1:1 to the ALS cases for age (± 5 years) and for gender. Determinations of Se compounds and of the heavy metals was performed using high pressure liquid chromatography (HPLC) coupled with inductively coupled plasma - dynamic reaction cell - mass spectrometry (FI-ICP-DRC-MS) according to methodologies previously established and described by one of the coauthors (B.M.).

[a]Including the inorganic and organic categories, albumin-bound selenium and the unknown forms

Results

We found higher levels of inorganic selenium species in the CSF of cases compared with controls. Conversely, the organic selenium forms were increased among controls, and this was particularly true for selenoprotein-P. Total selenium, determined in cases, was lower than that determined in controls, since the levels of the inorganic forms merged to the 'uncertain' species, greatly exceed the higher levels detected in ALS patients for the inorganic forms (Table 1).

The correlation between inorganic and organic selenium and the single selenium compounds were generally similar in the ALS and control groups. Relative risk (RR) of ALS directly correlated with selenite content in both conditional and unconditional logistic models, while ALS risk was inversely associated with most organic selenium species. In multivariate analyses adjusting for the other categories of selenium compounds, the associations were generally stronger than in crude analyses (Table 2).

Table 1. Distribution of different selenium species in ALS cases and controls (µg/l)

Selenium compound		centile 5th	centile 50th	centile 95th	mean	Difference (95% CI)	P-value
Se-SELENITE	controls	0	0.026	0.180	0.041	-0.021 (-0.049-0.007)	0.134
	cases	0	0.051	0.169	0.062		
Se-SELENATE	controls	0	0	0.089	0.012	0.001 (-0.012-0.013)	0.921
	cases	0	0	0.089	0.011		
Se-HSA	controls	0	0.087	0.258	0.102	-0.045 (-0.100-0.010)	0.104
	cases	0	0.127	0.359	0.147		
SELENOPROTEIN-P	controls	0.036	0.856	8.121	1.508	0.859 (-0.014-1.732)	0.054
	cases	0.093	0.577	2.196	0.649		
SELENOMETHIONINE	controls	0	0	0.305	0.052	0.050 (-0.026-0.125)	0.193
	cases	0	0	0	0.003		
GLUTATHIONE PEROXIDASE-BOUND Se	controls	0	0.021	0.164	0 .044	0.007 (-0.014 0.029)	0.503
	cases	0	0.026	0.120	0.036		
THIOREDOXIN-REDUCTASE-BOUND Se	controls	0	0.050	0.389	0.086	0.0263 (-0.014-0.067)	0.199
	cases	0	0.043	0.214	0.060		
TOTAL INORGANIC Se	controls	0	0.034	0.180	0.052	-0.021 (-0.053-0.012)	0.205
	cases	0	0.059	0.277	0.073		
TOTAL ORGANIC Se	controls	0.053	1.084	8.284	1.792	0.885 (-0.004-1.773)	0.051
	cases	0.287	0.765	2.508	0.907		
TOTAL Se [a]	controls	0.140	1.100	8.380	1.918	0.927 (-0.012-1.865)	0.053
	cases	0.320	0.765	2.660	0.991		

[a]ancluding the inorganic and organic categories, and the unknown forms

Table 2. RR with 95% confidence intervals (95% CI) of ALS associated with one-unit increased in CFS (µg/l) of Se species from logistic regression analysis

Selenium compound	Conditional logistic regression model RR	Conditional logistic regression model 95%CI (P-value)	Unconditional logistic regression model[b] RR	Unconditional logistic regression model[b] 95%CI (P-value)
Se-SELENITE	1.6	0.8-3.4 (0.184)	1.8	0.8-4.0 (0.163)
Se-SELENATE	0.9	0.2-4.2 (0.925)	0.9	0.2-4.8 (0.908)
Se-HSA	1.3	0.9-2.0 (0.150)	1.4	0.9-2.2 (0.120)
GLUTATHIONE PEROXIDASE-BOUND Se	0.02	4.87*e^{-07}-797.9 (0.468)	0.00	6.2e^{-7}-403.8 (0.423)
SELENOPROTEIN-P	0.4	0.11-1.13 (0.079)	0.4	0.16-1.10 (0.078)
THIOREDOXIN-REDUCTASE - BOUND Se	0.1	0.00-7.76 (0.239)	0.03	0-10.1 (0.244)
METHIONINE-BOUND Se	0.00	7.52*e^{-10}-1059.6 (0.325)	0.00	5.77*e^{-10}-2490.9 (0.365)
INORGANIC Se				
Crude	1.4	0.8-2.7 (0.253)	1.5	0.8- 3.0 (0.234)
Adjusted for organic selenium	1.8	0.8-3.7 (0.138)	1.9	0.9-4.1 (0.110)
ORGANIC Se				
Crude	0.5	0.2-1.1 (0.083)	0.5	0.3-1.1 (0.074)
Adjusted for inorganic selenium	0.4	0.2-1.0 (0.061)	0.5	0.2-1.0 (0.046)
TOTAL Se	0.5	0.3-1.1 (0.076)	0.6	0.32-1.06 (0.076)

[a] Increases of 1 µg/l except for selenite, HSA-bound selenium and inorganic selenium (100 µg/l)
[b] Adjusting for age and gender

Table 3. Distribution of different selenium species in ALS cases and controls aged ≥ 45 years (µg/l)

Selenium compound		median	mean	P-value
Se- SELENITE	controls	0.023	0.032	0.015
	cases	0.059	0.072	
Se- SELENATE	controls	0	0.008	0.410
	cases	0	0.014	
Se-HSA	controls	0.082	0.091	0.037
	cases	0.145	0.161	
SELENOPROTEIN-P	controls	0.877	1.881	0.040
	cases	0.577	0.639	
SELENOMETHIONINE	controls	0	0.055	0.310
	cases	0	0.003	
GLUTATHIONE PEROXIDASE-BOUND Se	controls	0.021	0.042	0.808
	cases	0.027	0.038	
THIOREDOXIN-REDUCTASE-BOUND Se	controls	0.043	0.059	0.729
	cases	0.050	0.064	
TOTAL INORGANIC Se	controls	0.030	0.040	0.017
	cases	0.072	0.086	
TOTAL ORGANIC Se	controls	1.16	2.13	0.049
	cases	0.81	0.92	
TOTAL Se	controls	1.33	2.26	0.055
	cases	0.93	1.02	

[b]Including the inorganic and organic categories, albumin-bound selenium and the unknown forms

The same differences were more pronounced in older patients and in females (Table 3 and 4). On the converse, not substantial differences in Cd, Hg and Pb concentrations emerged (Table 5).

Table 4. Relative risks (RR) with 95% confidence intervals (95% CI) of amyotrophic lateral sclerosis associated with one-unit increasea in CSF inorganic and organic selenium content in subjects aged ≥ 45 years

Selenium compound	Conditional logistic regression model RR	Conditional logistic regression model 95%CI (P-value)	Unconditional logistic regression model[b] RR	Unconditional logistic regression model[b] 95%CI (P-value)
Se-SELENITE	3.8	1.1-13.6 (0.039)	4.4	1.2-15.8 (0.023)
Se-HSA	1.8	0.9-3.4 (0.088)	1.8	1.0-3.4 (0.045)
SELENOPROTEIN-P	0.1	0.0-0.8 (0.030)	0.3	0.1-1.1 (0.073)
INORGANIC Se	3.2	1.0-10.0 (0.048)	3.4	1.1-10.0 (0.029)
ORGANIC Se	0.3	0.1-1.1 (0.064)	0.5	0.2-1.1 (0.103)
TOTAL Se	0.3	0.1-1.1 (0.083)	0.6	0.3-1.1 (0.107)

[a] Increases of 1 µg/l except for selenite, HSA-bound selenium and inorganic selenium (100 µg/l)
[b] Adjusting for age and gender

Table 5. Distribution of different selenium species in ALS cases and controls (ng/l)

Heavy metal		median	mean	P-value of the difference
Pb	controls	125	710.2	0.455
	cases	155	470.3	
Hg	controls	216.5	416.0	0.732
	cases	195.5	358.9	
Cd	controls	68.3	74.29	0.026
	cases	35.9	47.60	

[a]Adjusting for gender

Conclusion

These results support the hypothesis that inorganic selenium may trigger the neurodegenerative process characterizing ALS. The lower levels of Se-containing enzymes in ALS patients may indicate a deficiency of antioxidant response against free-radicals damage. Instead, the results do not support an involvement of three heavy metals, Cd, Hg and Pb, in ALS etiopathogenesis. However, caution must be used when inferring etiological clues from analytical results in patients affected by a severe disease such as ALS, and in hospital-referred controls. Further research on the involvement of Se in ALS etiology is clearly warranted.

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