Iron and Parkinson's disease: A systematic review and meta-analysis

GIOVANNI MOSTILE, CALOGERO EDOARDO CICERO, LORETTA GIULIANO, MARIO ZAPPIA and ALESSANDRA NICOLETTI

Department of Medical Sciences, Surgical and Advanced Technologies 'G.F. Ingrassia', University of Catania, I-95123 Catania, Italy

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Abstract. A possible association between iron serum levels and Parkinson's disease (PD) using a meta-analytic approach was evaluated. A systematic MEDLINE search was conducted to identify published observational, case-control studies dealing with the association between iron blood levels and PD. In both groups, iron blood levels were extracted as means and standard deviations to calculate the standardized mean differences (SMDs) with 95% confidence intervals (CIs). Heterogeneity of selected studies was investigated. Then, a meta-analysis was performed applying a random effects model. Possible causes of bias were also examined. A meta-regression analysis was finally conducted to investigate whether associations varied according to specified confounding factors. Of 155 studies detected by the research strategy, a total of 23 case-control studies with full available data were selected based on the adopted criteria. A small, around zero, overall SMD of -0.052 (95% CI, -0.303-0.2) was estimated, indicating no substantial differences between groups among selected studies. High heterogeneity among studies was detected (I^2=91.42%; p<0.001). By performing a meta-regression analysis considering single available demographic, geographical and clinical covariates, no significant association was detected. Based on our systematic revision and meta-analysis of available case-control studies, there was not sufficient evidence supporting a possible significant association between iron serum levels and PD as compared to controls. Principal reasons should be sought in the elevated methodological heterogeneity we found among available studies. A particular attention should be paid on bias and confounding effects to limit heterogeneity among studies and to facilitate the summary of results.

Correspondence to: Professor Alessandra Nicoletti, Department of Medical Sciences, Surgical and Advanced Technologies 'G.F. Ingrassia', Via Santa Sofia 79, I-95123 Catania, Italy
E-mail: anicolet@unict.it

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Introduction

Parkinson's disease (PD) is a neurological disorder with complex pathogenesis implicating both environmental and genetic factors. Epidemiological evidence suggests that chronic exposure to heavy metals such as iron, lead, manganese and their combinations can be associated with an increased risk of developing PD, since they could accumulate in the substantia nigra and generate oxidative stress (1).

Iron is an important microelement implicated in normal neuronal functioning as well as in several metabolic processes. Iron is first introduced in the body through various food products as iron salts or haemoglobin and it is then absorbed in the intestinal mucosa. Once in the enterocytes, iron is then excreted through transporter proteins (ferroportin and haephestin) in the bloodstream where it circulates attached to transferrin (2). After passing through the blood-brain barrier, iron enters the brain.

Non-physiological accumulation of iron in specific regions of the brain has been associated primarily with a heterogeneous group of diseases known as neurodegeneration with brain iron accumulation (NBIA) (3), but it has also been associated with Alzheimer's disease (4), amyotrohpic lateral sclerosis (5) and PD (6). Iron accumulation may exerts its pathogenic activity through the increase of reactive oxygen species (ROS) that then cause a wide array of damage to intracellular proteins, but there is also evidence of other mechanisms unrelated to ROS production, such as the promotion of apoptotic processes and the interaction with pathological protein aggregates found in these diseases (7).

PD is associated with extensive involvement of iron, with most extensive deposition in substantia nigra and lateral globus pallidus, as well as in dopaminergic neurons. The sources of increased iron should be searched in one or all of the following: homeostatic dysregulation, dysregulation of molecules involved in the intra and extracellular distribution of iron and aging (8).

Limited data are available concerning the levels of iron in serum/blood in PD and their relationship with the pathological process. It is not clear whether the alterations in metal homeostasis may be a cause or consequence in the pathology of the disease. However, whether metals are primary risk factors or their imbalances are consequences of pathological

mechanisms, changes in metal ion concentration may upset the whole element homeostasis, resulting in significant imbalances in element levels in the whole system (serum, cerebrospinal fluid and brain) (9). Even more, the effect of heavy metals, including iron, derived from environmental sources such as contaminated atmosphere or food/drinking water on the pathogenesis of PD and their relationship with their serum levels remain still difficult to investigate (10).

Few epidemiological studies have evaluated the possible association between serum/plasma levels of iron and PD and controversial results have been reported, probably due to methodological limitations. On this ground, we conducted a systematic review and a meta-analysis of the literature to better evaluate the possible relationship between iron and PD.

We evaluated a possible significant association between iron serum levels, expression of metal exposure or result of pathological processes related to the disease, and PD as compared to controls, using a meta-analytic approach of available case-control studies.

Materials and methods

Literature search. A systematic MEDLINE search was conducted by a medical investigator without time or language restriction, to identify published observational, case-control studies dealing with the association between iron blood levels and PD. Combined text words and Medical Subject Headings (MeSH) terminology were used. Specifically, to detect available study evaluating serum trace elements in PD including iron, the following search key words and boolean operators were entered in PubMed as search strategy: (metal* OR element* OR iron OR silicium OR nickel OR copper OR selenium OR zincum OR manganese OR chromium OR mercury) AND (blood or serum or plasma) AND Parkinson AND control*. Titles were scanned for relevance, identifying papers requiring further consideration. For the systematic research, a period up to September 2016 was considered.

Study selection. For the study selection, the following eligibility criteria were used considering PD as the outcome and iron blood level as exposure: i) the presence of a control group; ii) average values per group of iron blood levels together with information about methods used for the metal detection in the blood/serum and unit of measure adopted; iii) the sample sizes adopted. As additional criteria: iv) information about methods and criteria used for case-finding and control selection; v) information on demographical and geographical covariates; vi) the presence of a group-matching method by age and/or gender adopted in the selected case-control studies (if not directly available, it was subsequently tested using proper statistics); and vii) the presence of clinical information for cases characterization (i.e., disease stage or duration, motor status severity). The search results were independently assessed by a second reviewer. Disagreements were resolved through consensus among reviewers.

Data extraction and collection. The following data were extracted to be recorded in an ad hoc created collecting form: author, year of publication, measure unit adopted for the iron blood level, geographical information, setting, sample size, clinical-demographical characteristics and iron blood levels. Continuous variables were expressed as mean ± standard deviation (SD) and min-max range, categorical variables as frequency and percent values. Specifically, iron blood levels in both groups, since continuous data, were extracted as means and SDs to calculate the standardized mean differences (SMDs) with 95% confidence intervals (CIs).

Synthesis. Heterogeneity of selected studies was investigated using the forest plot as standardized method to display meta-analysis results and the I^2 statistic (11). To estimate the association between iron blood levels and PD as compared to controls, we then performed a meta-analysis applying a random effects model as more conservative approach than its fixed effect counterpart, assuming that, in addition to sampling variation, the true effect varies between studies. In this case, the conclusion estimate is considered as the mean effect assuming that the true study effects vary (12). Possible causes of bias were also examined. Presence of publication bias or, in the presence of small studies, or bias due to low methodological quality (12), was tested graphically using the funnel plot and implemented by the fail-safe N calculation using the Rosenthal approach, in order to estimate the number of additional 'negative' studies that would be needed to increase the P-value for the meta-analysis to ~0.05 (13). A meta-regression analysis was also conducted to investigate whether associations varied according to specified confounding factors, in particular demographic, geographical and clinical covariates, representing possible source of heterogeneity in observational studies (12). In the case of analysis based on one covariate, either the Z-test or the Q-test (equal to Z^2) was used to assess its relationship with effect size and thus regression model validity (14).

Results

Of 155 studies detected by the research strategy, a total of 23 case-control studies with full available data were selected based on the adopted criteria (period, 1992-2016) (Table I).

The studies were carried out in different countries [12 (52.2%) studies in Asiatic countries, of which five in China; eight (34.8%) studies in Europe, of which three in Italy and four in North-European countries; three (13%) studies in the American continent, of which two in the USA].

In 21 (91.3%) of the selected studies, cases selection was hospital-based, while in the other two studies it was community-based or based on registries. Adopted sample sizes for the cases group varied among studies, with a broad range of variation (mean ± SD, 66±52; min-max range, 13-238). The percentage of men in PD samples was on average 57.5±11.5 (min-max range, 37.8-92.3), with an average male-to-female ratio of 1.8±2.4 (min-max range, 0.5-12). Only 15 studies (65.2%) provided information on the adopted standardized diagnostic criteria for the cases selection (UK PD Society Brain Bank Clinical diagnostic criteria) (15).

Clinical information of cases were only partially provided by the adopted studies. Information on PD patients' disease duration were available in 11 (47.8%) studies, with an average value per group of 5.3±2.8 (min-max range, 2-9.5 years). Disease stage using standardized tool (the Hoehn and Yahr

Table I. Case-control studies on blood/serum iron levels in PD and controls (CTR). Iron detection methods and iron levels among groups (N=23).

Study, year (ref.)	Detection method	Measure unit	PD (N)	PD iron level (mean ± SD)	CTR (N)	CTR iron level (mean ± SD)
Chen and Shih, 1992 (21)	Unknown	μg/dl	15	95.53±33.5	30	102.5±32.5
Takahashi *et al*, 1994 (22)	PIXE	μg/ml	13	1.69±0.61	14	1.64±0.72
Logroscino *et al*, 1997 (23)	Atomic absorption graphite furnace micromethod	μg/dl	104	28.3±11.6	352	33.9±15.2
Jiménez-Jiménez *et al*, 1998 (24)	AAS	mg/l	37	1.01±0.33	37	0.95±0.3
Tórsdóttir *et al*, 1999 (25)	Colorimetric test with ferrozine ascorbic acid	μmol/l	33	16±4.25	33	16±4.5
Forte *et al*, 2004 (26)	ICP-AES	μg/l	26	1318±481	13	1136±393
Hedge *et al*, 2004 (9)	ICP-AES	μmol/ml	27	0.02±0.004	25	0.023±0.009
Qureshi *et al*, 2006 (27)	AAS	mg/ml	17	1.02±0.11	21	1.16±0.05
Annanmaki *et al*, 2007 (28)	Spectrophotometric reaction with ferrozine	μmol/l	40	18.2±5.5	29	20.5±6.3
Squitti *et al*, 2007 (29)	AAS	unknown	65	91.1±18.1	52	84.5±18.1
Gellein *et al*, 2008 (30)	HR-ICP-MS	μg/l	33	1275±551	99	1146±463
Ahmed and Santosh, 2010 (31)	ICP-AES and ICP-MS	μg/dl	45	110.4±0.6	42	123±8
Fukushima *et al*, 2010 (10)	ICP-AES	μg/ml	82	2±0.83	82	1.5±0.78
Fukushima *et al*, 2011 (32)	ICP-AES	μg/ml	71	1.95±0.85	71	1.44±0.77
Madenci *et al*, 2012 (33)	Unknown	unknown	60	74.6±29.3	42	74.8±27.1
Farhoudi *et al*, 2012 (34)	Unspecified biochemical methods	mg/dl	50	70.22±25.18	50	67.62±39.53
Fukushima *et al*, 2013 (35)	ICP-AES	μg/ml	58	2.1±0.84	81	1.51±0.78
Zhao *et al*, 2013 (36)	Fast sequential atomic absorption spectroscopy	μg/l	238	1656±749	302	1470±648
Kumudini *et al*, 2014 (37)	ICP-MS	ng/ml	150	554.4±123.8	175	421.7±126.1
Hu *et al*, 2015 (38)	ELISA	nmol/ml	102	4.124±1.064	31	4.192±1.054
Costa-Mallen *et al*, 2015 (39)	Unknown	μg/100 ml	128	83.28±29.46	226	94±34.14
Madeiros *et al*, 2016 (40)	Standard methods on *cobas mira*	μg/dl	40	67.5±18.89	46	78±18.15
Mariani *et al*, 2016 (41)	*Ferene*	μg/dl	92	79±34	112	86.1±34.9

Takahashi *et al*: 'untreated PD' and 'elderly controls' have been selected (age difference, p=0.915); Logroscino *et al*: matching done, but no data available; Tórsdóttir *et al*: SD estimated from range; Forte *et al*: PD HY stage, 1-3. 'Serum' (not 'blood') concentrations were selected; Hedge *et al*: only 'early PD group' considered: best age- (p=0.287) and gender- (p=0.991) matching; Qureshi *et al*: PD in 'on' phase selected: best age-matching (p=0.08); Squitti *et al*: two regions, only 'Valcamonica' region selected for PD and CTR (highest N); Gellein *et al*: preclinical PD group has been selected. Date for PD age, 1997; Fukushima *et al*: PD, 'no depression' group was selected; age- (p=0.711) and gender- (p=0.632) matched; Hu *et al*: PD, no RBD group. HY and disease duration as median (range). Mean and SD were estimated; Madeiros *et al*: medians and ranges provided. Means and SD were estimated. CTR from Santa Maria, same state; Mariani *et al:* medians and ranges provided. Means and SD were estimated. Age and gender matching (p<0.001). PD, Parkinson's disease; PIXE, Particle-Induced X-ray Emission; AAS, atomic absorption spectrophotometer; ICP-AES, inductively coupled plasma atomic emission spectrometry; HR-ICP-MS, high resolution inductively coupled plasma mass spectrometry; ELISA, enzyme-linked immunosorbent assay.

scale) (16) was provided in three (13%) studies (average value per group of 2±1; min-max range, 1-2.9). Standardized information on patients' motor status using the Unified PD Rating Scale (UPDRS) (17) total score was provided in seven (30.4%) studies, with an average value per group of 32.8±11.4 (min-max range, 18.8-45.1) (Table II).

Concerning the control groups, selection was hospital-based in 19 (82.6%) studies, while in the other three studies it was community-based and in a single study based on registries. An age and gender group-matching strategy was adopted in 16 (69.6%) studies, while a group-matching only by age was adopted in five (21.7%) studies. One study did not adopt any group-matching strategy. No data were provided in one study (Table III).

Iron serum levels (using different units of measure) in both groups and the different methods of detection adopted are shown in Table I. A meta-analysis was performed on all the 23 studies included. Results are summarized as forest plot in Fig. 1. A small, around zero, overall SMD of -0.052 (95% CI, -0.303-0.2) was estimated, indicating no substantial differences between groups among selected studies. I^2 statistic revealed high heterogeneity among studies (I^2=91.42%; p<0.001); it means that ~91% of the observed variance comes from real differences between studies and can potentially be explained by study-level covariates.

Asymmetry in funnel plot was consistent with the presence of publication bias in favor of positive results, showing also that studies were principally allocated in bottom of the

Table II. Case-control studies on blood/serum iron levels in PD and controls (CTR). PD group characteristics.

Study, year (ref.)	PD (N)	PD source	Diagnostic criteria	PD men (N, %)	PD M/F ratio	PD age (mean ± SD)
Chen and Shih, 1992 (21)	15	Hospital	Not specified	Not specified	Not specified	Not specified
Takahashi *et al*, 1994 (22)	13	Hospital	Not specified	Not specified	Not specified	61.2±10.3
Logroscino *et al*, 1997 (23)	104	Community	UK Brain Bank	Not specified	Not specified	Not specified
Jiménez-Jiménez *et al*, 1998 (24)	37	Hospital	UK Brain Bank	14 (37.8)	0.61	65.7±8.8
Tórsdóttir *et al*, 1999 (25)	33	Hospital	Not specified	18 (54.5)	1.2	67±8.5
Forte *et al*, 2004 (26)	26	Hospital	UK Brain Bank	24 (92.3)	12	64.9±10.8
Hedge *et al*, 2004 (9)	27	Hospital	Not specified	14 (51.8)	1.08	57.1±5.2
Qureshi *et al*, 2006 (27)	17	Hospital	UK Brain Bank	10 (58.8)	1.43	70±15
Annanmaki *et al*, 2007 (28)	40	Hospital	UK Brain Bank	23 (58)	1.35	60.8±6.5
Squitti *et al*, 2007 (29)	65	Hospital	Not specified	34 (52.3)	1.1	67.9±7.1
Gellein *et al*, 2008 (30)	33	Hospital	UK Brain Bank	16 (48.5)	0.94	61.1±9.1
Ahmed and Santosh, 2010 (31)	45	Hospital	Not specified	26 (57.8)	1.37	57.6±9.1
Fukushima *et al*, 2010 (10)	82	Hospital	UK Brain Bank	47 (57.3)	1	63.9±9.4
Fukushima *et al*, 2011 (32)	71	Hospital	UK Brain Bank	41 (57.7)	1.37	63.7±9.7
Madenci *et al*, 2012 (33)	60	Hospital	UK Brain Bank	33 (55)	1.22	68.5±9.2
Farhoudi *et al*, 2012 (34)	50	Hospital	Not specified	28 (56)	1.27	64.5±10.2
Fukushima *et al*, 2013 (35)	58	Hospital	UK Brain Bank	36 (62.1)	1.64	64.3±9.4
Zhao *et al*, 2013 (36)	238	Hospital	UK Brain Bank	121 (50.8)	1.03	66.6±11.3
Kumudini *et al*, 2014 (37)	150	Hospital	Not specified	107 (71.3)	2.49	55.7±10.6
Hu *et al*, 2015 (38)	102	Hospital	UK Brain Bank	47 (46.1)	0.46	56.3±13.4
Costa-Mallen *et al*, 2015 (39)	128	Registry	UK Brain Bank	88 (68.7)	2.2	69
Madeiros *et al*, 2016 (40)	40	Hospital	UK Brain Bank	18 (45)	0.82	69.95±12.3
Mariani *et al*, 2016 (41)	92	Hospital	UK Brain Bank	62 (67.4)	2.07	70±11.25

Table III. Case-control studies on blood/serum iron levels in PD and controls (CTR). CTR group characteristics.

Study, year (ref.)	CTR (N)	CTR source	CTR men (N, %)	CTR M/F ratio	CTR age (mean ± SD)	Age matching	Gender matching
Chen and Shih, 1992 (21)	30	Hospital	Not specified	Not specified	Not specified	Not specified	Not specified
Takahashi *et al*, 1994 (22)	14	Hospital	Not specified	Not specified	60.8±8.9	Yes	Not specified
Logroscino *et al*, 1997 (23)	352	Community	Not specified	Not specified	Not specified	Yes	No
Jiménez-Jiménez *et al*, 1998 (24)	37	Hospital	16 (43.2)	0.76	62.4±17.8	Yes	Yes
Tórsdóttir *et al*, 1999 (25)	33	Hospital	Not specified	Not specified	Not specified	Yes	Yes
Forte *et al*, 2004 (26)	13	Hospital	6 (46.1)	0.86	63.8±13.7	Yes	No
Hedge *et al*, 2004 (9)	25	Hospital	13 (52)	1.08	55.4±6.4	Yes	Yes
Qureshi *et al*, 2006 (27)	21	Hospital	13 (61.9)	1.63	62±11	Yes	Yes
Annanmaki *et al*, 2007 (28)	29	Hospital	13 (45)	0.81	60.2±5.1	Yes	Yes
Squitti *et al*, 2007 (29)	52	Hospital	32 (61.5)	1.6	71.1±8.5	Yes	Yes
Gellein *et al*, 2008 (30)	99	Community	48 (48.5)	0.94	Not specified	Yes	Yes
Ahmed and Santosh, 2010 (31)	42	Hospital	25 (59.9)	1.47	55.6±3.2	Yes	Yes
Fukushima *et al*, 2010 (10)	82	Hospital	47 (57.3)	1	63.6±9.3	Yes	Yes
Fukushima *et al*, 2011 (32)	71	Hospital	41 (57.7)	1.37	63.4±9.7	Yes	Yes
Madenci *et al*, 2012 (33)	42	Hospital	22 (52.4)	1.1	66.9±8.3	Yes	Yes
Farhoudi *et al*, 2012 (34)	50	Hospital	25 (50)	1	63.5±9.8	Yes	Yes
Fukushima *et al*, 2013 (35)	81	Hospital	47 (58)	1.38	63.7±9.4	Yes	Yes
Zhao *et al*, 2013 (36)	302	Hospital	153 (50.7)	1.03	65.6±12.2	Yes	Yes
Kumudini *et al*, 2014 (37)	175	Hospital	120 (70.6)	2.18	53.7±10.9	Yes	Yes
Hu *et al*, 2015 (38)	31	Hospital	Not specified	Not specified	Not specified	Yes	Not specified
Costa-Mallen *et al*, 2015 (39)	226	Registry	104 (46)	0.85	62.6	Yes	Not specified
Madeiros *et al*, 2016 (40)	46	Community	19 (41)	0.7	62.3±10.2	Yes	Yes
Mariani *et al*, 2016 (41)	112	Hospital	40 (35.8)	0.56	62±14	No	No

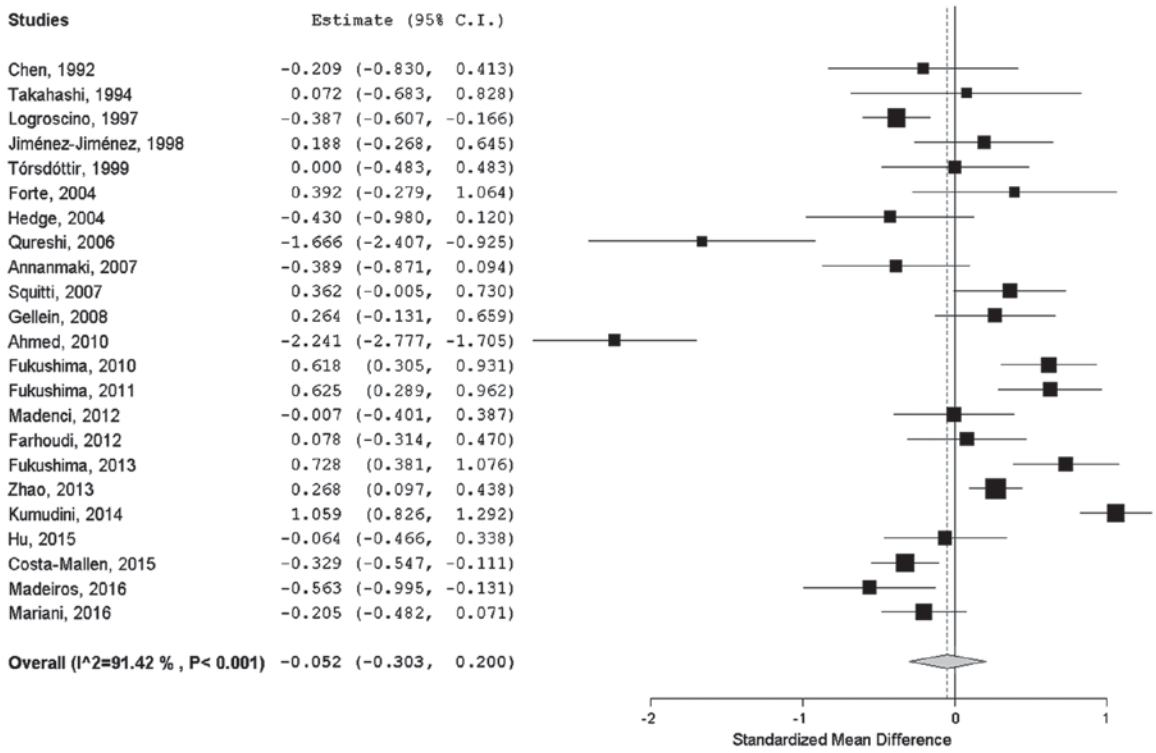

Figure 1. Iron serum concentration in PD and controls (N=23). Forest plot with SMDs (95% CI) (random-effect method). PD, Parkinson's disease; SMDs, standardized mean differences; CI, confidence interval.

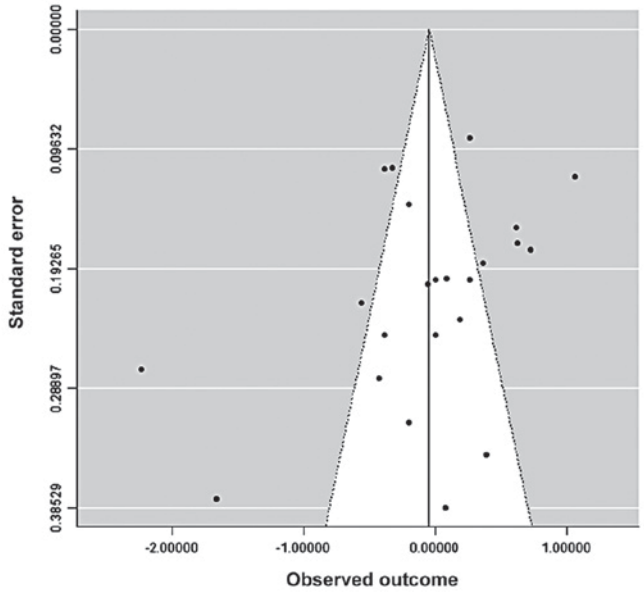

Figure 2. Funnel plot (N=23).

graph indicating a broad range of standard error of the effect measures among studies due to their small sample size (Fig. 2). The fail-safe N calculation was equal to zero (observed significance level, 0.319; target significance level, 0.05).

A meta-regression analysis was then performed considering single demographic, geographical and clinical covariates using a random-effects model. The regression coefficient for latitude was 0.003, which means that every one degree of latitude corresponds to an increase of 0.003 units in effect size, even if this was not statistically significant (Z=0.457, Q=0.209, p=0.648). Regression coefficients for longitude and altitude were respectively 0.002 (Z=1.175, Q=1.38, p=0.240) and 0 (Z=0.721, Q=0.52, p=0.471), both not statistically significant (Fig. 3).

Demographical covariates, based on the assumption that most of studies were group-matched by age and gender, the regression coefficient for PD age was -0.01 (N=21, Z=-0.269, Q=0.072, p=0.788) while for the PD male-to-female ratio was 0.049 (N=20, Z=0.66, Q=0.436, p=0.509), both not statistically significant (Fig. 3).

When we looked at the clinical characteristics of selected groups of cases, we found a regression coefficient for disease duration of 0.035 (N=11, Z=0.607, Q=0.368, p=0.544) and for the UPDRS total score of 0.017 (N=7, Z=1.28, Q=1.64, p=0.201), both not statistically significant.

Discussion

Iron and its deregulated homeostasis have been proposed to have a role in the pathogenesis of PD because of its pro-oxidants characteristics that may lead to ROS generation via Fenton and Haber-Weiss reactions. However, epidemiological evidence concerning the possible association between iron and PD remains still controversial.

In this systematic revision and meta-analysis, we searched for a possible association between serum iron levels and PD, as compared to controls. Based on selected case-control studies, we did not find a significant pooled mean difference between groups. Our results are in agreement with a previous meta-analysis by Mariani *et al* (18) demonstrating no variation of metal concentrations in serum between PD patients and healthy controls (SMD, -0.45; 95% CI, -0.98-0.08), probably due to

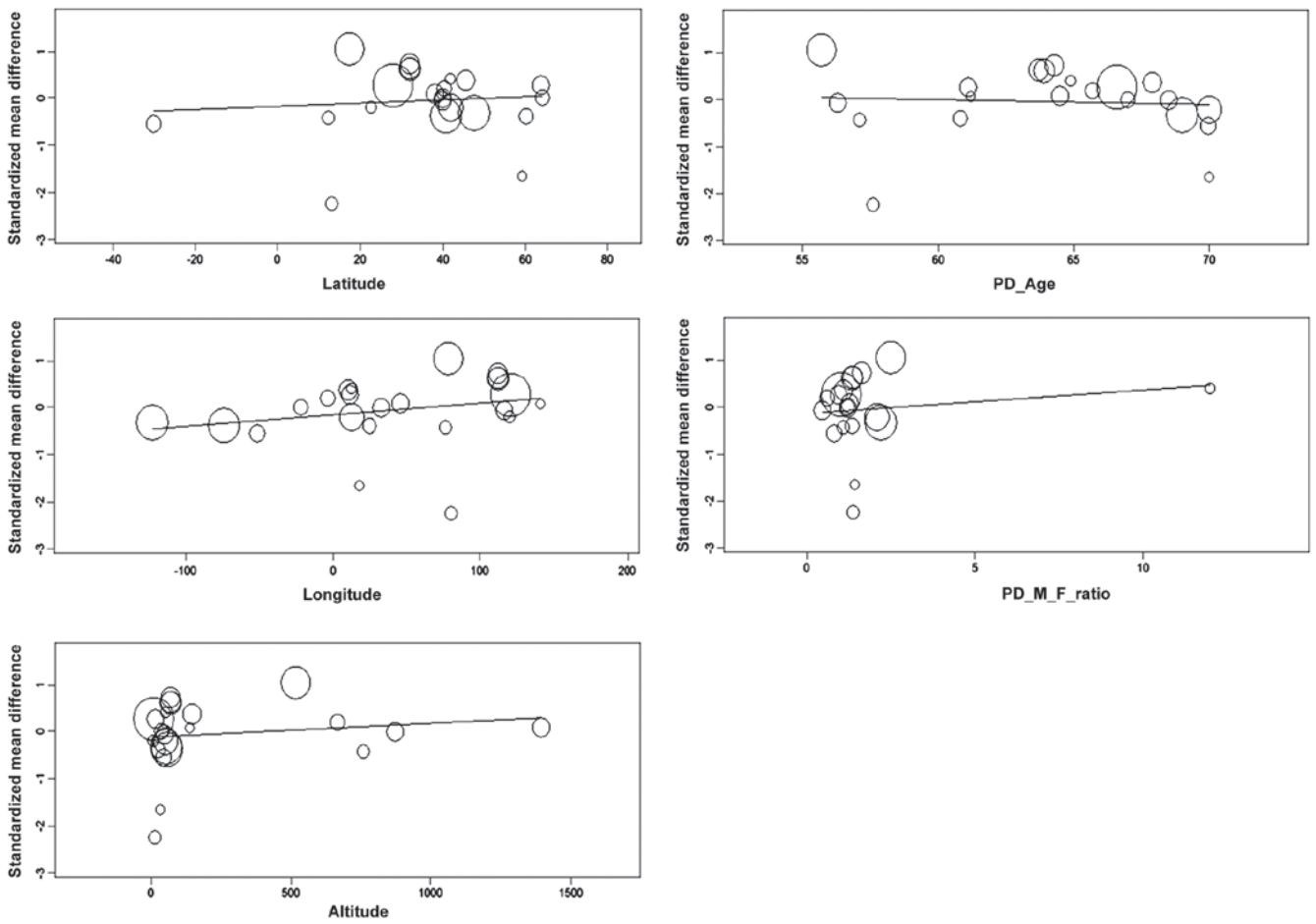

Figure 3. Meta-regression plots. Geographical and demographic covariates (random-effect method).

the high heterogeneity among evaluated studies (I^2=93.4%; p<0.001). Evaluation of a second meta-analysis based on 11 selected studies showed instead overall higher iron serum levels in PD patients when compared to controls (SMD, 0.97; 95% CI, 0.18-0.37), even though a significant heterogeneity was also found among studies (I^2=96.5%; p<0.001) (19).

We confirmed a high level of heterogeneity among evaluated studies, as expression of the overall small-sampled, methodologically-limited studies available in literature which are insufficient to provide practical evidence; for instance, most of the selected studies had a hospital-based design, which does not permit to exclude a possible selection bias. Moreover, the presence of publication bias could have lead to an underestimation of possible negative results.

Heterogeneity among studies can be related to several issues: the different approaches in finding and selection of cases and controls; the lack of an adequate matching strategy between groups; the use of an inadequate sample size for obtaining statistically-relevant results; the different methods used for iron detection in blood samples; the lack of corrected analysis for possible confounders including comorbidities affecting iron serum levels. These issues justified the choice of a random-effects approach for meta-analysis of data.

We performed a meta-regression analysis considering single available demographic, geographical and clinical covariates, all potential confounders affecting our pooled results. However, no significant association was detected.

Limits of the present meta-analysis are the same as those related to other meta-analysis performed using observational, case-control studies as target. In particular, the appropriate control of confounding factors is of fundamental importance in the analysis and interpretation of observational studies. The presence of other types of bias, for example recall bias, should represent additional concerns (12). Even more, we focused our selection on studies evaluating serum iron levels and not other biological fluids (i.e., urine or cerebrospinal fluid) or tissues (i.e., hair), limiting results interpretation. Furthermore, it should be underlined that in case-control studies evaluating a possible association between metals and PD by detecting levels of metals in biological fluids, these measurements often show just the actual iron homeostasis, lacking a clear history of exposure to high levels of this metal (20). Finally, even if a systematical approach has been used for the search strategy, we cannot exclude that some data were missed affecting the study results.

In conclusion, based on our systematic review and meta-analysis of available case-control studies, we can state there are still not sufficient evidence supporting higher or lower serum levels of iron in PD patients as compared to controls, assuming this may be related to metal exposure or pathological processes in such subjects. Principal reasons should be sought in the elevated methodological heterogeneity we found among

available studies. A particular attention should be paid on bias and confounding effects to limit heterogeneity among studies and to facilitate the summary of the results.

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