Trace Metals in Patients with Parkinson's Disease: A Multi-Center Case-Control Study of Nigerian Patients

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Abstract: *Background*: The roles of environmental factors in the etiologic consideration of Parkinson's disease (PD) need investigation and clarification, especially in sub-Saharan Africa where genetic mutations are rare. Trace metals toxicity has been associated with pathogenesis of neuro-degeneration including Parkinson's disease.

Methods: PD patients presenting to three tertiary health facilities located in the south-west, south-south and central Nigeria were studied and compared with age and sex matched controls from the same regions using a protocol containing a structured questionnaire, diagnostic criteria based on the United Kingdom Parkinson's Disease Society brain bank and atomic absorption spectrophotometry method for analysis of plasma trace metals – copper, zinc, magnesium, manganese and iron.

Findings: Sixty eight consecutive PD patients with a mean age of 65.7±7.29 years and a male preponderance (Male (46)/Female (22) = 2.1:1) had significantly elevated trace metals (namely copper, zinc, magnesium and iron) compared to controls (P<0.001). The means of the trace metals' levels for the PD and controls were – Copper 51.8 and 14.7µmol/l, Magnesium 3.35 and 2.06mEq/l, Manganese 15.6 and 14.4µmol/l, Iron 78.5 and 17.4µmol/l and Zinc 88.7 and 19.3µmol/l respectively. There was no significant difference in level of manganese between PD patients and controls, though elevated in PD patients residing in the southern part of the country the difference between the PD patients and controls in central region (P=0.29) was insignificant.

Conclusion: The findings of this study suggested a possible role for trace metals toxicity in the pathogenesis of Parkinson's disease. There is however need for further studies to elucidate the specific roles of these trace metals in the etiology of PD.

Keywords: Parkinson's disease, trace metals, copper, zinc, manganese, iron, neuro-degeneration.

INTRODUCTION

The etiology of Parkinson's disease (PD) has remained elusive despite the flurry of researches and publications available on it. It is the second most common neurodegenerative disorder and occurs in 2% of the population over the age of 60 years [7]. It affects over 1million people in North America, occurring commonly after the age of 65years [1, 23]. Age is the single most consistent risk factor and, with the increasing age of the general population of sub-Saharan African sub-continent, the prevalence of Parkinson's disease is expected to rise steadily in future [19,23]. It is associated with significant mortality and by the year 2040, neurodegenerative diseases are projected to surpass cancer as the second most common cause of death [20]. It is important to note that apart from age, a family history of PD is the strongest predictor of an increased risk [31]. Reduction in risk has been associated with cigarette smoking, especially among the young-onset patients [11,22,34].

Parkinson's disease is relatively rare among Asians and African blacks. An age-adjusted prevalence rate of 67 per 100,000 was reported by Schoenberg *et al.* among Nigerians above 39 years in a door-to-door survey of a rural community in South west Nigeria [30]. This low prevalence has been attributed to the absence of environmental factors associated with urbanization which predispose to the development of PD. Though the etiology of Parkinson's disease remains unknown, there are several hypotheses which have been

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postulated. The genetic hypothesis witnessed a major breakthrough with the identification of 2 distinct mutations in the α-synuclein gene (SNCA) 4q [6]. Mutations have been identified in the gene among rare families with dominant PD suggesting that aggregation of this protein in Lewy bodies is probably crucial to pathogenesis of the disease. There have also been reports on linkage to chromosome 2p13 [8]. Dominant PD has however been more commonly linked with leucine-rich repeat kinase 2 (LRRK2) while early onset autosomal recessive PD has been found in association with parkin gene, DJ-1 and PINK1 [23] although these genetic mutations are rare in Nigerians [25].

The peak incidence of PD in Nigerians is in the sixth decade of life [25]. At onset of disease, 72% of Nigerians were over the age of 50 and 90% were over the age of 40. The mean age of PD was reported to be 55.6 years with the peak frequency at onset in the seventh and eighth decades [30].

The major environmental factors linked to etiology of Parkinson's disease (PD) are consumption of well water [28], exposure to herbicides and pesticides (especially among forestry workers) [5,15,29] and drugs like 1,2,3,4, methylphenyl tetrahydropyridine (MPTP), a meperidine analogue which caused parkinsonism in young American drug addicts in the 1980s [2,35]. The delayed onset, progressive Parkinsonism after the pandemic of encephalitis lethargica strengthens the environmental causation theory [37]. The concept of apoptosis in PD etiology is not universally acceptable, but the critical components of nigral degeneration in PD, which include mitochondrial dysfunction, oxidative stress, actions of excitotoxins, deficient neurotrophic support and underlying immune mechanisms, have been described. For instance, the MPTP toxicity is due to the inhibition of complex I (NADH-ubiquinone oxido-reductase) of the mitochondrial election-transport chain with consequent failure of energy generation and cell death. In PD there is a 30-40% reduction in complex I activity in the substantial nigra pars compacta as well as a lesser defect in other tissues [14,18].

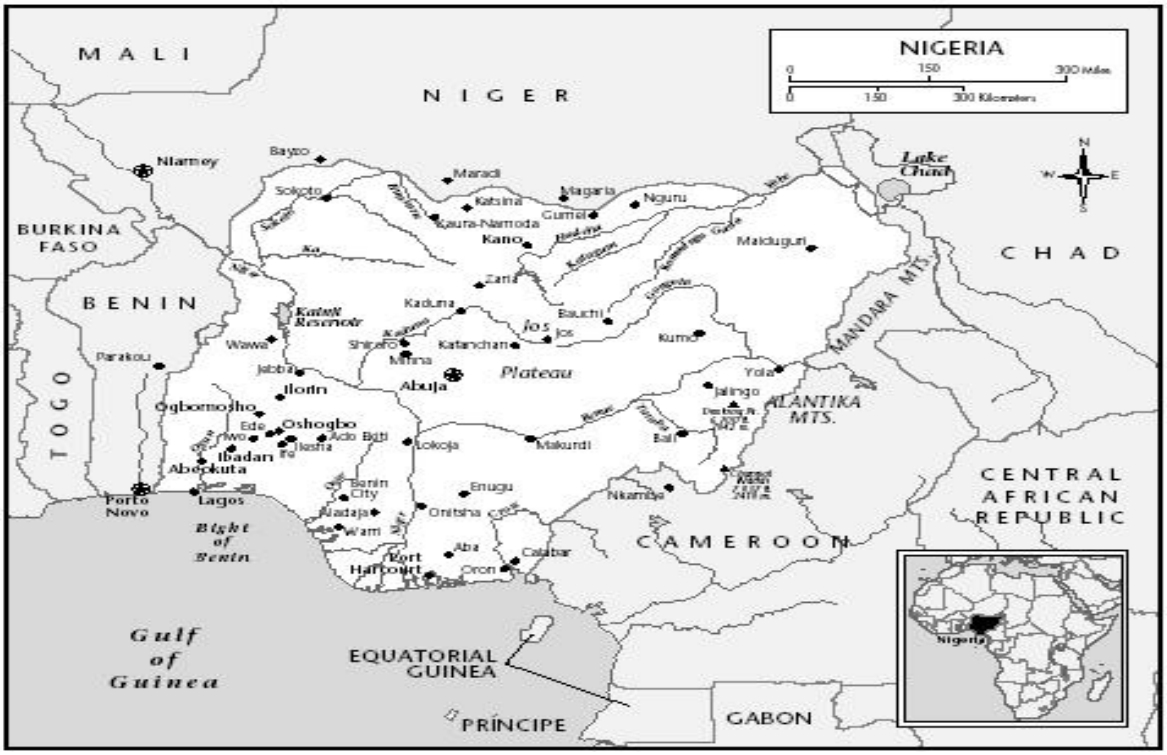
Trace elements are normally present in very low concentrations in the body. Trace metal analysis of body fluids reflects the body burden, or internal dose, of the individual being tested i.e. it examines the amount of the metal that has actually entered the body and remained there. This is called biological monitoring or 'bio-monitoring'. So bio-monitoring is the measurement of a substance or its metabolites in the body fluids or tissues in an attempt to assess the potential health risk the substance may induce [10,27].

Alterations in levels of trace metals have been linked to the aetio-pathogenesis of neurodegeneration, including PD [9]. There are reports implicating manganese, aluminum, lead, cyanides, copper, zinc and iron in neuro-degeneration [32,36,38]. Trace metals are metallic elements with high atomic weight and their toxicities can cause damage to living organisms at very low concentrations, e.g. copper, zinc, cadmium, lead and manganese. Heavy metals are natural components of the earth's crust, and cannot be degraded or destroyed. Small quantities enter the body *via* food, drinking water and air. Toxicity occurs when there is increase in the levels of these metals in the human tissues. As trace elements some of these heavy metals are essential to the maintenance of the body's metabolism. PD has been linked to heavy metal toxicity, often from bioaccumulation, resulting in progressive degeneration of neurons due to disturbance of normal body metabolic reactions [4].

This case-control cross sectional study was undertaken to assess the plasma levels of trace metals in patients with PD in three tertiary health facilities situated in major urban cities in the middle belt, south west and south-south Nigeria and determine if there is any association between the occurrences of PD and trace metals in Nigerians residing in urban areas. This is a unique setting as there has been no previous study that assesses the levels of trace metals among Nigerians with PD.

METHODS

This was a cross-sectional case-control study involving three tertiary health facilities which serve as major referral centers for neurological diseases in their respective geopolitical regions in cosmopolitan Nigerian urban cities situated in middle belt (Ilorin), south west (Ile-Ife) and south-south (Benin City) (see Figure **1**). The study was conducted between January 2007 and December 2009 with the aid of a protocol which included a questionnaire to obtain the demographic and clinical data of all patients presenting to the neurology clinics, diagnostic criteria for PD and laboratory techniques for trace metals analysis.. Demographic information obtained included age, sex, domicile, level of education and occupation. Clinical information obtained included age at onset of symptoms, duration of symptoms, family history of PD, use of drugs or substances, consumption of well water, previous history of central nervous system infections,



NIGERIA'S GEOPOLITICAL ZONES

South West	Ekiti, Lagos, Osun (Ile-Ife), Ondo, Ogun, Oyo
South East	Asia, Anambra, Ebonyi, Enugu, Imo
South-South	Akwa-Ibom, Bayelsa, Cross-River, Delta, Edo (Benin City), Rivers
North Central	Benue, FCT, Kogi, Kwara (Ilorin), Nasarawa, Niger, Plateau
North East	Adamawa, Bauchi, Borno, Gombe, Taraba, Yobe
North West	Kaduna, Katsina, Kano, Kebbi, Sokoto, Jigawa,, Zamfara

Figure 1: Map of Nigeria showing the study sites (Benin City, Ife, Ilorin).

history of head injury and other medical conditions like hypertension, diabetes mellitus, renal and cerebrovascular diseases.

The diagnosis of PD was made using the United Kingdom Parkinson Disease Society Brain Bank (UKPDS Brain Bank) Criteria [33] and level of severity was determined with the Hoehn and Yahr staging criteria [12]. Ethical approval was given by the respective hospital ethics committees of the three study centers. Informed consent was obtained from all study participants.

Sample Size Estimation

The minimum sample size of patients required for the study was 28, calculated based on the Kish method [17].

$$n = \frac{z^2\ pq}{d^2} = \frac{1.96^2\ \text{x}\ 0.0006\ \text{x}\ 0.9994}{(0.009)^2} = 28.44$$

n = the desired sample size (when population is greater than 10,000)

z = the standard normal deviation, usually set at 1.96, which corresponds to the 95% confidence level

p = proportion of patients with PD estimated at 59 per 100,000[30]

q = 1- p

d = absolute deviation from p% that will be tolerated

Patients' Selection

Sixty-eight consecutive patients with confirmed clinical diagnosis of PD based on the UKPDS Brain Bank criteria were recruited for the study at the three centers comprising twenty patients from middle belt (Ilorin), twenty from south west (Ile-Ife) and twenty-eight from south-south (Benin City) – Figure **1**. The

patients were compared with sixty age- and sex-matched controls selected from the same centers (20 controls from each of the study centers). The criteria aided in the exclusion of differential diagnoses of PD. Patients with history of oral consumption of dietary supplements containing trace elements were excluded.

Laboratory Analysis of Trace Metals

About 10mls of venous blood was obtained from each study participant. The blood samples were collected by venepuncture into EDTA-anticoagulated bottles and centrifuged at 3000rpm for 10minutes to separate plasma from cells. Plasma was separated into another clean plastic container before analysis. Plasma levels of the trace metals were determined with flame atomic absorption spectrophotometer (AAS) using a direct method as described by Kaneko (1999) [16]. The AAS was done twice and then double-checked once. This method is based on the principle that atoms of the metals when aspirated into AAS, vaporized and absorbed light of the same wavelength as that emitted by the metal when in the excited state. Adequate precautions were taken to minimize exposure of the blood specimens to atmospheric air and to avoid contact with rubber, wood and paper products. Contact with metal surfaces was also avoided except the steel needle used for venipuncture.

Statistical Analysis

The data was analyzed with Statistical Package for the Social Sciences (SPSS) version 16. The demographic and clinical characteristics data were presented using descriptive statistics of frequency distribution, means and percentages. The categorical data from the PD patients and controls were analyzed for statistically significant differences with one-way analysis of variance and chi-square distribution. The differences in plasma levels of trace metals between patients and controls at each center and the overall sample from all centers were analyzed for statistical significance using Student t test. The level of significance was taken as p less than 0.05.

RESULTS

The mean age of the sixty-eight patients was 65.7 ± 7.29 years with a male preponderance (Male (46)/Female (22) = 2.1:1). The details of the demographic data from the three centers are presented in Table **1**. Using one-way analysis of variance and chi-square distribution for age and sex respectively, there were no significant differences in the age (F=0.496; P=0.61 for patients, F=0.134; P=0.88 for controls) and sex (X^2 = 0.246; P=0.88 for patients, X^2 = 0.574; P=0.75 for controls) of the patients and controls recruited at the three study sites (P>0.05). The mean duration of symptoms before presentation was 3.6±0.51 years.

Clinical Characteristics of Patients

The Hoehn and Yahr severity staging [12] revealed 45 patients (70.3%) in stages 1 and 2, while 17(26.6%) had postural involvement and two patients were bedridden. There were no differences in the clinical features of the male and female patients (P=0.87). Ten of the patients (15.6%) gave a positive family history of PD. The main non motor features observed were autonomic and psychiatric (depression). Excessive day time somnolence occurred in a male patient and this led to early retirement from his place of work. A female patient attempted suicide due to severe depression and this necessitated urgent psychiatric intervention.

Trace Metals Analysis

The details of analysis of the trace metals are presented in Table **2**. The PD patients had significantly

Table 1: Comparison of Age and Sex of Study Participants at the Three Health Facilities

Variables	Benin City (south-south) PD patients (N=28)	Benin City (south-south) Controls (N=20)	Ile-Ife (south west) PD patients (N=20)	Ile-Ife (south west) Controls (N=20)	Ilorin (middle belt) PD patients (N=20)	Ilorin (middle belt) Controls (N=20)	Total (PD patients) (N=68)	Total (controls) (N=60)
Mean age (SD)	66.8 (6.67)	61.2 (5.33)	62.7 (10.32)	61.9 (10.25)	60.5 (8.88)	62.3 (6.77)	65.7 (7.29)	61.5 (8.23)
*P values	0.157		0.488		0.201		0.167	
Sex								
Male	18	12	14	12	14	14	46	38
Female	10	8	6	8	6	6	22	22
**P values	0.772		0.741		1.269		0.710	

Table 2: Trace Metals Levels in Parkinson's Disease Patients and Controls

Trace metals	South-south (Benin) N 48 (P=28;C=20)	South west (Ile Ife) N 40 (P=20;C=20)	Middle Belt (Ilorin) N 40(P=20; C=20)	Total N 128 (P=68; C=60)	Normal range of laboratory values
Cu (µmol/l)					11 – 28 µmol/l
Patients	73.5±14.98	36.4±5.63	28.9±3.71	51.8±5.37	
Controls	10.4±5.17	12.7±6.68	11.5±4.63	14.7±5.33	
P values	P<0.0001	P<0.0001	P<0.0001	P<0.0001	
Mg (mEq/l)					1.8 – 2.5 mEq/l
Patients	3.70±1.31	3.63±2.91	2.90±1.53	3.35±1.34	
Controls	1.80±0.93	2.16±1.09	1.36±0.90	2.06±0.92	
P values	P<0.001	P<0.05	P<0.05	P<0.001	
Mn ((µmol/l)					9 – 20 µmol/l
Patients	21.0±4.2	12.22±4.87	20.24±15.52	15.63±8.32	
Controls	10.10±0.03	26.47±14.54	24.83±11.21	14.43±9.21	
P values	P<0.0001	P=0.0002	P=0.2904	P=0.4401	
Fe (µmol/l)					9 -27 µmol/l
Patients	104.3±16.7	129.14±32.52	20.69±2.65	78.46±23.81	
Controls	25.7±7.21	12.01±3.32	9.18±4.22	17.43±5.31	
P values	P<0.0001	P<0.0001	P<0.0001	P<0.0001	
Zn (µmol/l)					8 – 20 µmol/l
Patients	97.6±4.52	50.67±24.78	101±11.33	88.7±13.83	
Controls	25.7±3.67	8.99±3.55	46±21.13	19.27±9.48	
P values	P<0.0001	P<0.0001	P<0.0001	P<0.0001	

Cu – Copper, Mg – Magnesium, Mn – Manganese, Fe – Iron, Zn – Zinc, P- patients, C – controls, N – sample size, P – patients, C- controls.
Level of significance - P<0.05.

elevated trace metals namely copper, zinc, magnesium and iron compared to age and sex matched controls. The level of manganese was elevated in PD patients residing in the southern part of the country but there was no difference between the PD patients and controls in middle belt. There was no significant difference in the manganese plasma levels among the PD patients from all the three study sites when compared to the controls (P=0.44).

DISCUSSION

This study showed the significant elevations in plasma trace metals namely copper, magnesium, iron and zinc in Nigerian patients with PD residing in urban cities without significant difference in plasma manganese levels between the patients and controls, although significant elevations in plasma manganese were observed among PD patients residing in southern Nigeria but not among those living in the central Nigeria. Several reports have alluded to the roles of trace metals toxicity in the pathogenesis of neuro-degeneration [3,4,9,33,38]. The presence of elevated concentrations in our patients raised the likelihood of association between trace metals toxicity and PD among Nigerian Africans. This is the first study to examine the levels of trace metals in PD among Nigerian Africans.

The clinical profile of our PD patients is consistent with observations from previous studies from Nigeria [26,28]. The severity of the disease at presentation is probably due to late presentation as observed in this study corroborating an earlier study from south western Nigeria [26].

A study in the United States demonstrated increase risks of developing PD with exposure to high levels of lead, manganese or copper in urban areas corroborating previous laboratory research which showed that these metals caused changes in tissue similar to those seen in PD and some observational studies that found that some people exposed to these metals in their occupation developed clinical syndromes similar to PD. The study suggested that these metals may interfere with the brain's ability to scavenge toxic radicals and may also induce or accelerate the formation of clumps of protein found in people who have neurodegeneration [36]. The lack of significant elevation in level of manganese obtained in this study did not support this observation. An

important differential diagnosis of PD in the presence of high manganese level is managanism which manifests with a Parkinsonian-like syndrome of gait disturbance, tremors, expressionless or masked-like facies and psychiatric disturbance. This syndrome is commonly seen following occupational exposure among miners, arc welders and dry battery manufacturers [13,21].

Evidence also suggests that there is an excess of reactive oxygen species and increased oxidative stress in PD. Elevated iron levels has been demonstrated in the pars compacta in PD patients, this being an important factor in causing oxidative stress. However, increase iron and reduced complex I activity are absent in patients with incidental lewy body disease. A reduction in level of reduced glutathione is evident at the early state. The metabolism of endogenous dopamine may result in the formation of toxic by-products, contributing to heightened state of oxidative stress in PD [4]. Iron is an important constituent of succinate dehydrogenase as well as a part of heme of hemoglobin, myoglobin and the cytochromes and its accumulation has been related to neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [4,9,38].

Copper is an essential substance to life as it is present in many enzymes involved in oxidation (tyrosinase, ceruloplasmin, amine oxidase and cytochrome oxidase). It is a micronutrient useful for normal functioning of the hematologic and neurologic systems such that in low concentration can render the brain susceptible to free radical damage and in toxic concentration can affect cellular metabolism [32,33,38]. Zinc is a divalent metal which forms an integral component of about 200 metallo-enzymes including carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, glutamic dehydrogenase; as well as hormones like thymulin, testosterone, prolactin and somatomedin. Excess zinc can act as pro-oxidants thus increase oxidative stress and enhance neuro-degeneration causing an increased risk of PD [9,38]. The plasma levels of these trace metals (i.e. copper, zinc and iron) were increased in our PD patients. It is difficult to conclude that the etiology of PD in these patients is due to these trace metals. However, the significant elevations of trace metals in these PD patients could have contributed to the evolution of the neuro-degeneration though further investigation is needed to ascertain this.

Manganese is an essential cofactor in humans for antioxidant activity of enzymes like superoxide dismutase, but toxic in large amounts causing damage to the liver, lungs, vascular endothelium and brain [32,36,38]. It is used industrially in steel making, iron making and in fertilizers [38]. It has been implicated in the etiology of striatal degeneration [36]. Its concentrations are highest in the globus pallidus, striatum, thalamus and substantia nigra [32]. Inhaled manganese is transported directly to the brain before it can be metabolized in the liver, resulting in permanent brain damage characterized by tremors, difficulty in walking and facial muscle spasms [21,32]. Our study however did not show any difference between the patients and the controls but the levels of manganese were significantly elevated in PD patients living in the south west and south-south unlike the observation in the central part of the country. The reason for this disparity is not immediately obvious from our study.

In conclusion our study has demonstrated the presence of elevated plasma levels of trace metals – copper, zinc, magnesium and iron in patients with Parkinson's disease residing in the central, south west and south-south Nigeria. The elevation of plasma manganese level observed in the southern regions was not observed in the central part of the country. There is need for further study to elucidate the role of these trace metals in the etiology of PD in our environment where genetic mutations have been demonstrated to be rare.

AUTHORS' ROLES

Ogunrin AO, Komolafe MA Sanya EO and Osubor C were involved in the conception and organization of the research project. Ogunrin AO, Komolafe MA, Sanya EO, Osubor C, Ajose OA, Akande AA and Mosaku SK executed the project. Ogunrin AO and Komolafe MA designed and executed the statistical analysis. Ogunrin AO wrote the first draft. All the authors participated in the review and critique of the manuscript.

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The authors have no financial disclosures or conflicts of interest to declare.

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REFERENCES

[1] Bennett DA, Beckett LA, Murray AM, *et al*. Prevalence of parkinsonian signs and associated mortality in a community population of older people. N Engl J Med 1996; 334: 71-76. http://dx.doi.org/10.1056/NEJM199601113340202

[2] Calne DB. Is idiopathic parkinsonism the consequence of an event or a process? Neurology 1994; 44: 5-10. http://dx.doi.org/10.1212/WNL.44.1.5

[3] Chandra RK. Micro-nutrients and immune functions. An overview. Annal New York Acad Sci 1990, 587: 9-16.

[4] Dexter DT, Carayon A, Javoy-Agid F, *et al*. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 1991; 114: 1953-75. http://dx.doi.org/10.1093/brain/114.4.1953

[5] Engel LS, Checkoway H, Keifer MC, *et al*. Parkinsonism and occupational exposure to pesticides. Occup Environ Med 2001; 58: 582-89. http://dx.doi.org/10.1136/oem.58.9.582

[6] Gasser T, Muller-Myhsok B, Wszolek ZK, *et al*. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet 1998; 18: 262-65. http://dx.doi.org/10.1038/ng0398-262

[7] Gasser T. Genetics of Parkinson's disease. Curr Opin Neurol 2005; 18(4): 363-69. http://dx.doi.org/10.1097/01.wco.0000170951.08924.3d

[8] Gasser T. Update on the genetics of Parkinson's disease. Mov Disord 2007; Suppl 17: S343-50. http://dx.doi.org/10.1002/mds.21676

[9] Gorell JM, Johnson CC, Rybick BA, *et al*. Occupational exposure to Manganese, Copper, Lead, Iron and Zinc and the risk of Parkinson's disease. Neurotoxicity 1999, 20(2/3): 239-49.

[10] Guidotti TL, McNamara J, Moses MS. The interpretation of trace element analysis in body fluids. Indian J Med Res 2008; 128: 524-32.

[11] Hellenbrand W, Seidler A, Robra BP, *et al*. Smoking and Parkinson's disease: a case –control study in Germany. Int J Epidemiol 1997; 26: 328-39. http://dx.doi.org/10.1093/ije/26.2.328

[12] Hoehn MM, Yahr MD. Parkinsonism onset, progression and mortality. Neurology 1967; 17(5): 427-42. http://dx.doi.org/10.1212/WNL.17.5.427

[13] Hu H. Poisoning, Drug Overdose and Envenomation. In 'Harrison's Principles of Internal Medicine' 16th ed. (Eds. Kasper DL, AS Fauci, DL Longo, E Braunwauld, SL Hauser, & JL Jameson) Chapter 376, pg. 2580, McGraw-Hill, New York, 2005.

[14] Jenner P, Olanow CW. Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 1996; 47: Suppl 3: S161-S170. http://dx.doi.org/10.1212/WNL.47.6_Suppl_3.161S

[15] Kamel F, Hoppin JA. Association of Pesticide Exposure with neurologic dysfunction and disease. Environ Health Perspect 2004; 112(9): 950-58. http://dx.doi.org/10.1289/ehp.7135

[16] Kaneko JJ. Clinical Biochemistry of Animal. 4th ed. J.J. Kaneko (ed.) Academic Press Inc. New York, 1999: pp. 932.

[17] Kish L. Survey sampling. New York: John Wiley and Sons; 1965.

[18] Lang AE, Lozano AM. Parkinson's Disease – First of two parts. N Engl J Med 1998; 339: 1044-53. http://dx.doi.org/10.1056/NEJM199810083391506

[19] Louis ED, Marder K, Cote L, Tang M, Mayeux R. Mortality from Parkinson disease. Arch Neurol 1997; 54: 260-64. http://dx.doi.org/10.1001/archneur.1997.00550150024011

[20] Lilienfeld DE, Perl DP. Projected neurodegenerative disease mortality in the United States, 1990-2040. Neuroepidemiology 1993; 12: 219-28. http://dx.doi.org/10.1159/000110320

[21] Montegomery EB Jr. Heavy metals and the etiology of Parkinson's disease and other movement disorders. Toxicol 1995, 97: 3-9. http://dx.doi.org/10.1016/0300-483X(94)02962-T

[22] Morens DM, Grandinetti A, Reed D, White LR, Ross GW. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology 1995; 45: 1041-51. http://dx.doi.org/10.1212/WNL.45.6.1041

[23] Morens DAM, Davis JW, Grandinetti A, Ross GW, Popper JS, White LR. Epidemiologic observations on Parkinson's disease: incidence and mortality in a prospective study of middle-aged men. Neurology 1996; 46: 1044-50. http://dx.doi.org/10.1212/WNL.46.4.1044

[24] Okubadejo N, Britton A, Crews C, *et al*. Analysis of Nigerians with apparently sporadic Parkinson disease for mutations in LRRK2, PRKN and ATXN3. PLoS One 2008; 3(10): e3421. http://dx.doi.org/10.1371/journal.pone.0003421

[25] Okubadejo NU. An analysis of genetic studies of Parkinson's disease in Africa. Parkinsonism Relat Disord 2008; 14: 177-82. http://dx.doi.org/10.1016/j.parkreldis.2007.08.006

[26] Okubadejo NU, Ojo OO, Oshinaike O. Clinical profile of parkinsonism and Parkinson's disease in Lagos, Southwestern Nigeria. BMC Neurology 2010; 10: 1. http://dx.doi.org/10.1186/1471-2377-10-1

[27] Paustenbach D, Galbraith D. Biomonitoring and biomarkers: exposure assessment will never be the same. Environ Health Persp 2006; 114: 1143-49. http://dx.doi.org/10.1289/ehp.8755

[28] Priyadarshi A, Khuder SA, Schaub EA, Priyadarshi SS. Environmental risk factors and Parkinson's disease: a metaanalysis. Environ Res 2001; 86: 122-27. http://dx.doi.org/10.1006/enrs.2001.4264

[29] Ritz B, Yu F. Parkinson's disease mortality and pesticide exposure in Carlifornia 1984-1994. Int J Epidemiol 2000; 292: 323-29. http://dx.doi.org/10.1093/ije/29.2.323

[30] Schonberg BS, Osuntokun BO, Adeuja AOG, *et al*. Comparison of the prevalence of Parkinson's disease in black populations in the rural United States and in rural Nigeria: Door-to-door community studies. Neurology 1988; 38: 645-46. http://dx.doi.org/10.1212/WNL.38.4.645

[31] Semchuk KM, Love JE, Lee RG. Parkinson's disease: A test of the multifactorial etiologic hypothesis. Neurology 1993; 43: 1173-80. http://dx.doi.org/10.1212/WNL.43.6.1173

[32] Sloot WN, Van der Sluys-Gelling, Crambsbergen JB. Selective lesions by manganese and extensive damage by iron after injection into rat striatum or hippocampus. J Neurochem 1994, 62: 205-16. http://dx.doi.org/10.1046/j.1471-4159.1994.62010205.x

[33] Taylor KSM, Counsell C. Is it Parkinson's disease, and if not, what is it? Pract Neurol 2006, 6: 154-65. http://dx.doi.org/10.1136/jnnp.2006.091835

[34] Tzourio C, Rocca WA, Breteler MMB, *et al*. Smoking and Parkinson's disease: an age-dependent risk effect? Neurology 1997; 49: 1267-72. http://dx.doi.org/10.1212/WNL.49.5.1267

[35] Vingerhoets FJG, Snow BJ, Tetrud JW, Langston JW, Schulzer M, Calne DB. Positron emission tomographic evidence for progression of human MPTP-induced dopaminergic lesions. Ann Neurol 1994; 36: 765-70. http://dx.doi.org/10.1002/ana.410360513

[36] Willis AW, Evanoff BA, Lian M, *et al*. Metal emissions and urban incident Parkinson Disease: A community Health Study of Medicare Beneficiaries by using Geographic Information Systems. Am J Epidemiol 2010; 172(12): 1357-63. http://dx.doi.org/10.1093/aje/kwq303

[37] Zhang Z-X, Roman GC. Worldwide occurrence of Parkinson's disease: an updated review. Neuroepidemiology 1993; 12: 195-208. http://dx.doi.org/10.1159/000110318

[38] Zotta P, Luchini R, Van Rensburg SJ, Taylor A. The role of metals in neurodegenerative processes: Al, Mn and Zn. Brain Res Bull 2003, 62(1): 15-28. http://dx.doi.org/10.1016/S0361-9230(03)00182-5

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