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ORIGINAL ARTICLE

Plasma Homocysteine Level and its Relationship to Clinical Profile in Parkinson's Disease Patients at the Lagos University Teaching Hospital

Taux d'homocystéine plasmatique et sa relation avec le profil clinique chez les patients la maladie de Parkinson à l'hôpital de Lagos Enseignement universitaire

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ABSTRACT

BACKGROUND: **Hyperhomocysteinaemia (HHcy) is as a long-term sequelum of levodopa therapy in Parkinson's disease (PD). Information on its frequency and effects in Africans with PD is sparse.**

OBJECTIVE: **To determine the frequency of HHcy and its relationship to clinical features of PD in African patients.**

METHODS: **Using a case-control design, 40 consecutively attending PD patients and 40 age- and gender-matched healthy volunteering controls were studied. Parkinson's disease cases were evaluated for disease and treatment characteristics, using the Unified Parkinson Disease Rating Scale (UPDRS) motor and activities of daily living scores and disease stage (Hoehn and Yahr scale). Fasting total plasma homocysteine (Hcy) was determined in all subjects. Hyperhomocysteinaemia was defined as a Hcy level above the 90th percentile for the controls.**

RESULTS: **Mean Hcy was 13.8 ± 5.4μmol/L in PD and 12.4 ± 3μmol/L in controls (P>0.05). Hyperhomocysteinaemia (Hcy > 16.26umol/L) occurred in nine (22.5%) PD patients (all on levodopa) and 6 (15%) controls (P>0.05). Mean duration of levodopa use was 92 ± 105.3 months in PD with HHcy compared to PD patients with normal Hcy 33.9 ± 33.2 (p < 0.05). Disease severity and disability were similar regardless of Hcy levels. None of current age, disease duration, Hoehn and Yahr stage, UPDRS scores, total levodopa dose and duration was independent predictor of homocysteine level.**

CONCLUSION: **There is increased occurrence of hyper-homocysteinaemia in Nigerian subjects with Parkinson's disease, receiving Levodopa. This hyperhomocysteinaemia is more common with prolonged use but appears to have no relationship with disease severity or disability. WAJM 2011; 30(5): 319–324.**

Keywords: **Parkinson's disease, homocysteine, Nigeria, severity, disability.**

RÉSUMÉ

CONTEXTE: hyperhomocystéinémie (HHcy) est comme un sequelum à long terme de traitement par la lévodopa dans la maladie de Parkinson (PD). Information sur sa fréquence et les effets chez les Africains avec PD est clairsemée.

OBJECTIF: Déterminer la fréquence des HHcy et sa relation avec les caractéristiques cliniques de PD chez les patients africains.

MÉTHODES: En utilisant une étude cas-contrôle, 40 patients parkinsoniens qui fréquentent consécutivement et 40 d'âge et le sexe des sujets sains contrôles volontaires ont été étudiés. Cas de maladie de Parkinson ont été évalués pour caractéristiques de la maladie et le traitement, en utilisant l'échelle Unified Parkinson Disease Rating (UPDRS) du moteur et les activités de la vie quotidienne des scores et le stade de la maladie (échelle de Hoehn et Yahr). Le jeûne homocystéine plasmatique totale (Hcy) a été déterminée dans toutes les matières. Hyperhomocystéinémie a été défini comme un niveau Hcy dessus du 90e percentile pour les contrôles.

RÉSULTATS: L'Hcy était de 13,8 ± 5.4mmol / L dans la MP et de 12,4 ± 3 mmol / L chez les témoins (P> 0,05). Hyperhomocystéinémie (Hcy> 16.26umol / L) a eu lieu dans neuf (22,5%) chez les patients parkinsoniens (tout sur la lévodopa) et 6 (15%) des contrôles (P> 0,05). La durée moyenne d'utilisation était de 92 ± lévodopa 105,3 mois en PD avec HHcy par rapport à des patients parkinsoniens avec Hcy normale 33,9 ± 33,2 (p <0,05). Gravité de la maladie et le handicap étaient similaires quel que soit taux de Hcy. Aucun âge actuel, durée de la maladie, de Hoehn et Yahr étape, les scores UPDRS, la dose de lévodopa et de la durée totale était prédicteur indépendant du niveau d'homocystéine.

CONCLUSION: Il est augmentée apparition de l'hyper-homocystéinémie chez les sujets nigérians atteints de la maladie de Parkinson, la réception lévodopa. Cette hyperhomocystéinémie est plus fréquente avec l'utilisation prolongée, mais semble avoir aucune relation avec la sévérité de la maladie ou le handicap. **WAJM 2011; 30(5): 319–324**

Mots-clés: maladie de Parkinson, l'homocystéine, le Nigeria, la gravité, le handicap.

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Abbreviations: ADL, Activities of Daily Living; **DA,** Dopamine agonists; **HY,** Hoehn and Yahr; **HHcy,** Hyperhomocysteinaemia; **Hcy,** Homocysteine; **LD,** Levodopa; **LUTH,** Lagos University Teaching Hospital; **MDS,** Movement Disorder Society; **PD,** Parkinson's disease; **SD,** Standard deviation; **tHcy,** total homocysteine; **UKPDS,** United Kingdom Parkinson's Disease Society; **UPDRS,** Unified Parkinson Disease Rating Scale; **UPDRS ADL,** Unified Parkinson Disease Rating Scale ADL score; **UPDRS M,** Unified Parkinson Disease Rating Scale Motor score; **UPDRS T,** Unified Parkinson Disease Rating Scale Total score

INTRODUCTION

Parkinson's disease (PD) occurs predominantly in the elderly and has a considerable impact on the quality of life.[1] Over the past decade, the risk of vascular disease (including ischemic stroke) in PD has become the focus of debate particularly with respect to the role of levodopa (LD) therapy.[2] Levodopa therapy has been associated with an increased frequency of elevated levels of the amino acid homocysteine (Hcy).[3,4] Elevated levels of Hcy are an emerging risk factor for stroke,[5,6] Alzheimer's disease,[7] and affective disorders;[8] all of which have the potential to worsen morbidity and consequently quality of life in the PD patient.

Homocysteine levels are significantly increased in levodopa-treated PD patients compared to untreated PD patients and age-matched controls.[9] In fact, total Hcy (tHcy) concentrations have been reported to be 30-80 per cent higher in levodopa-treated PD patients.[3,4,10,11] This study aimed at evaluating the frequency of HHcy in African PD cases in the Neurology Clinic of LUTH, and explores its relationship to their clinical profile.

SUBJECTS, MATERIALS, AND METHODS

Study Site and Recruitment Method

The study was conducted at the Out-patients Neurology Clinic of the Lagos University Teaching Hospital (LUTH) in Lagos State, South western Nigeria. The LUTH is a tertiary referral centre and has a wide catchment area including Lagos State, contiguous states and other distant states within Nigeria. In addition, referrals are routinely received from the west African subregion. The study duration was from March to September 2006. Approval of the study protocol was obtained from the Health Research and Ethics Committee of LUTH.

Consecutively attending and consenting patients with Parkinson's disease were enrolled into the study on fulfilment of the inclusion criteria. The PD cases included old attendees and new referrals. Control subjects were age- and gender-matched volunteers without any pre-existing neurological disorder, drawn from the general population.

Case Ascertainment

Parkinson's disease was diagnosed based on the universally accepted United Kingdom Parkinson's Disease (UKPDS) Brain Bank clinical diagnostic criteria.[12] To distinguish PD from other forms of parkinsonism, the criteria were strictly adhered to and thus excluded patients with signs and symptoms to suggest secondary parkinsonism. Specifically, the study excluded patients with any identifiable secondary cause (e.g. previous strokes), lack of asymmetry at onset, presence of atypical features (e.g. early and prominent dysautonomia, early or preceding cognitive impairment, cerebellar features, temporal relationship to drug or toxin exposure, bilateral symptoms at onset, lower body parkinsonism, muscle weakness and otherwise unexplained corticospinal tract signs). In addition, PD cases and controls with co-morbidities associated with increased Hcy levels such as chronic kidney disease, leukaemia, or medications that increase Hcy (e.g. thiazides and anticonvulsants) were excluded.

Clinical Assessment in Parkinson's Disease Cases

Parkinson's disease-related characteristics including disease severity and disability were documented in all cases. Evaluations were conducted in the "off" state (i.e. without medication effect). Each patient was assessed using the Unified Parkinson Disease Rating Scale (UPDRS) version 3.0[13] and the Hoehn and Yahr (HY) scale.[14] The UPDRS version 3.0 has four components as follows: I – mentation, behaviour and mood, II – Activities of Daily Living, III – Motor assessment, and IV – Complications of therapy. The relevant sections assessed for this study were ADL (for degree of disability) and Motor assessments (for disease severity). The total score obtainable in each of these sections is 52 and 108 respectively. For standardization, the assessments were conducted in accordance with instructions contained in the training video provided for this purpose by the Movement Disorders Society (Movement Disorders Society (MDS) Standardized training tools for UPDRS ADL scale[15] and the teaching tape for the motor section of the UPDRS.[16]

Disease severity was also assessed using the HY scale which is a 5-scale instrument that uses clinical description of motor features of parkinsonism to stage the disease. Stage 1 refers to unilateral signs, stage 2 – bilateral signs with no axial involvement, stage 3 – bilateral signs with axial involvement, stage 4 – severe symptoms though still independent, and stage 5 – wheelchair/bed bound.

Total daily levodopa (LD) dosages (in the preceding four weeks) were as documented in the case records and corroborated by the patients. Usage of LD was categorised as high (≥ 1000mg/24 hours) and low (<1000mg/24 hours) (based on the proposed threshold dose of LD at which HHcy occurs) for the purpose of analysis.[17] The duration of LD treatment was divided into long (≥ 24 months) and short (<24 months)[17] and the disease duration was divided into long (≥ 36 months) and short (<36 months).[18]

Homocysteine Estimation

Total plasma homocysteine was estimated from samples drawn after an overnight fast (≥ 12 hours). The blood samples were centrifuged within one hour of collection at 1,000 revolutions per minute using a Beckman table centrifuge. Plasma was stored at minus 20°C until batched analysis after every 20 samples. Total L-Hcy was assayed by the fluorescence polarisation immunoassay (FPIA) method of Schipchandler *et al,*[19] using an IMx® Abbot immunoassay instrument (Abbot Laboratories, Illinois, USA). For this method, bound Hcy (oxidized form) is reduced to free Hcy which is then enzymatically converted to S-adenosyl-L-Hcy (SAH). Total free Hcy is converted to SAH by the use of SAH hydrolase, and excess adenosine SAH hydrolase converts SAH to Hcy. Excess adenosine in the pretreatment solution drives the conversion of Hcy to SAH by the bovine SAH hydrolase. A minimum volume of 50 ul of plasma is used for the assay. Abnormal total Hcy was defined as any level above the 90th percentile in the distribution of Hcy in the control population for this study. Consequently, Hyperhomocysteinaemia (HHcy) was defined as total plasma Hcy level greater than 16.3umol/L.

Data Analysis

Data was analyzed using the SPSS version 17.0 statistical software. Numerical data are presented as means ± standard deviation (SD) and ranges and compared using parametric student's T-test. Categorical variables are presented as proportions and compared using non-parametric χ^2 test. Fisher exact test was used to compare data sets with any cell value less than 5. As recommended by the MDS Task force report on the HY staging scale, summary data for HY stage are presented as median values.[20]

RESULTS

Baseline, Discipline, Characteristics

The characteristics of the PD cases and controls are shown in Table 1 which also includes disease-specific variables relevant to the PD cases only. The mean age ± SD (years) in PD was 65.8 ± 9.8, comparable to that of controls (63.3 ± 10.8; P = 0.27). Gender was similarly distributed for cases and controls.

Of the PD cases on treatment 14 (40%) were on LD monotherapy, 10 (28.6%) were on LD in combination with dopamine agonists (DA) (bromocriptine), while 4 (11.4%) were on a combination LD, DA and an anticholinergic (trihexyphenidyl). 7(20%) of the PD cases on treatment were on a combination of LD and artane only. Overall 19(54.3%) of the PD cases had been on treatment for a long duration while 16 (45.7%) had been on treatment for a short duration.

The mean (SD) LD dose for those with PD for a longer duration (≥ 36 months) (n = 25) was significantly higher (737.0 ± 247.5 mg/24 hours) than that of PD of short duration (n = 10) (453.7 ±145.6) (P < 0.05).

Table 1: Characteristics of Parkinson Disease Patients and Controls

Variable	Parkinson disease N = 40	Controls N = 40
Age range	42 – 86	42 – 85
Mean age ± SD (years)	65.8 ± 9.8	63.3 ± 10.8
Gender distribution (Male: Female)	4 : 1	4 : 1
Mean ± SD duration of disease (months)	64.9 ± 49.6	NA
Median Hoehn & Yahr score	2	NA
Mean UPDRS total score ± SD	57.7 ± 25.0	NA
Mean UPDRS motor score ± SD	41.1 ± 17.6	NA
Mean UPDRS ADL score ± SD	13.6 ± 7.2	NA
Mean total plasma Hcy ± SD (umol/L)	13.8 ± 5.4	12.4 ± 3.3
Frequency of Hyperhomocysteinemia (%)	22.5	15.0

No difference was statistically significant between the two groups.

Table 2: Comparison of Clinical Variables in PD cases with and without Hyperhomocysteinaemia

Clinical Variable	PD with hyperhomocysteinemia N = 9	PD without hyperhomocysteinaemia N = 31
Disease Severity		
Median Hoehn and Yahr scores	2	2
Mean ± SD Hoehn & Yahr scores	2.2 ± 0.7	2.3 ± 1.1
Mean ± SD UPDRS (total) scores	57.1 ± 13.5	57.9 ± 27.7
Mean ± SD UPDRS (ADL) scores	12.6 ± 5.8	13.9 ± 7.6
Mean ± SD UPDRS (motor) scores	41.2 ± 8.6	41.1 ± 19.6
Treatment-related variables		
Mean ± SD Levodopa dose (mg/24hrs)*	672.2 ± 270.2	650.5 ± 256.7
Mean ± SD Levodopa treatment (months)*	92.0 ± 105.3†	33.9 ± 33.2
Mean ± SD Homocysteine (umol/L)	21.9 ± 3.0†	11.1 ± 2.9
Disease-related variable		
Mean age (years)	69.7 ± 7.3	64.9 ± 10.2
Disease duration ± SD (months)	88.0 ± 53.3	58.1 ± 47.2

**Mean LD dose and duration of LD treatment calculated for only patients receiving levodopa i.e. total of 9 PD with Hyperhomocysteinaemia and 26 PD without hyperhomocysteinaemia; †Difference statistically significant at p < 0.05.*

Homocysteine Levels in PD Cases and Controls

The mean Hcy level for the PD cases was 13.8 ± 5.4 µmol/L comparable to control Hcy level of 12.4± 3.3 µmol/L (P = 0.17). Based on the definition of HHcy, 9 (22.5%) of the PD cases had HHcy compared with 6 (15%) of the controls. The difference was not statistically significant (χ^2 = 0.74, p = 0.39). There was no statistical difference regarding gender either within the cases or the controls.

Table 2 shows the clinical variables in PD cases with and without HHcy.

The mean Hcy level for PD cases on high dose levodopa was 13.5 µmol/L ± 6.7. This was lower than the mean Hcy level ± SD for the cases on low dose levodopa which was 14.3 µmol/L ±5.5 (P>0.05). Mean Hcy level was higher in PD cases on LD for long duration (15.44 ± 6.56 months) compared to those on treatment for a short duration (12.15 ± 3.54 months), but the difference was not significant (P = 0.09). Mean Hcy was also higher in PD cases with longer disease duration (≥3 years) (14.61 µmol/L ± 6.04) compared to those with shorter disease duration (<3 years) (12.27 ± 3.98 µmol/L; P= 0.27).

The frequency of HHcy in the PD cases was 9 (25.7%) cases on levodopa compared to none of the 5 cases who were LD naïve (P=0.57). Eight (42.1%) of the cases on LD for a long duration (>24 months) compared to 1/16 (6.3%) of the cases on the drug for a short duration had elevated Hcy levels (P < 0.05).

Of those on high dose LD treatment (≥1g/day) 4(25%) had HHcy compared to

Table 3. Regression Analysis of Putative Determinants of Plasma Homocysteine Levels

Variable	Coefficient	Std. Error	t	P
Age (years)	–0.0360	0.7518	0.05	0.48
Age at onset (years)	0.1477	0.7305	0.20	0.42
Disease duration (months)	0.0117	0.0549	0.21	0.42
H & Y stage	0.1298	2.1763	0.06	0.48
UPDRS ADL score	0.0499	0.5315	0.09	0.46
UPDRS M score	0.2598	0.3614	0.72	0.24
UPDRS T score	–0.1958	0.3610	0.54	0.30
Levodopa dose /24hrs	–0.0005	0.0036	0.13	0.45
Levodopa duration	0.0275	0.0222	1.24	0.11

8(25.8%) of those on <1g/day (P =0.44). Other comparisons regarding HHcy in PD are shown in Table 2.

Multivariate regression analysis however failed to show any relationship between the disease and treatment variables and homocysteine value as shown in Table 3.

DISCUSSION

In recent years, the prospect and implications of Hyperhomocysteinaemia (HHcy) as a complication of treatment of PD with levodopa (the mainstay of PD treatment in most instances) has been a subject of interest. This is particularly important because despite the association with worse disease severity and disability and adverse cardiovascular events, HHcy is potentially treatable using simple, available and affordable vitamin supplements such as folate and cyanocobalamin.[21, 22]

The present study found the frequency of HHcy in our PD cases as 22.5% (25.7% in those on LD and 0% in the LD naïve PD), compared to the lower frequency (although statistically insignificant) of 15% in controls. The documented frequency of HHcy in PD varied widely, and can be attributed mainly to variations in the cut-off value used to define HHcy. The defining cut-offs have been selected in relation to the mean Hcy of controls in some studies,[4, 23] whereas Hcy above the 90th percentile in controls,[24] and Hcy levels associated with increased cardiovascular risk in longitudinal studies have also been utilised.[25–27] Lamberti *et al*[25] utilized plasma Hcy levels above 12μmol and reported a significantly higher frequency of 80% in their cases compared to 25% in controls. However, in another report from the same study group,[26] in which plasma Hcy >14umol/L was the defining limit the frequency of HHcy was 70% for PD cases on LD only. O'Suilleabhain *et al*[27] also used a cut off value of 14umol/L and reported the frequency of HHcy in PD cases only (no controls) as 32%. The lower frequency in our study may thus reflect the higher cut off value used. However, our findings still lend credence to the aetiologic role of LD in the causation of HHcy in PD.

Another factor that could potentially contribute to levels of Hcy in PD would be the dose of LD. A threshold level of 1000 mg has been suggested as being strongly correlated with the occurrence of HHcy in PD. In our study, the average daily LD dose of ~660mg was lower than this threshold and may contribute to the lower frequency of HHcy.[17] It may not be entirely explanatory however as it falls within the range (235mg/day to 790mg/day) reported for other studies in which higher frequencies of HHcy were reported (refs).[4,9,26–28]

The mean plasma Hcy levels in our PD cases did not significantly vary from that of controls, a finding that has been reported previously from other patient populations in Nigeria.[24] We also note that the high level of plasma Hcy seen in controls in this study (12.4umol/L) is similar to previous reports in other studies using African population,[24,29,30] and may partly explain the lack of significance noted in our study.

The higher levels of Hcy in our controls may be due to differences in the nutritional composition and habits, although the role of pharmacogenomic factors cannot be excluded.

In contrast, studies from Caucasian populations have often reported significantly higher Hcy levels in PD cases on treatment (LD) compared to controls.[11,23,28,31]

Our study did not demonstrate any relationship between disease severity or disability in comparing PD with and without HHcy as has been documented in some studies.[27,32] In a prospective study of 97 PD cases, O'Suillebhain *et al* found insignificantly higher UPDRS motor scores both at baseline[27] and at 2-year follow-up[32] in the PD cases with HHcy. Todorovic *et al,*[18] in their comparison of LD treated and LD naïve PD cases also did not find any correlation between the serum total Hcy levels and disease severity or disability both for the PD cases on LD and those that were LD naive. However they only studied PD cases with mild disease (HY stages 1 and 2). The association between HHcy and disease severity/duration/disability may be explained by the fact that patients with advanced PD necessarily require more dopamine replacement typically derived from administration of higher doses of LD. This is buttressed by our results showing a significant correlation between LD dose and duration of PD. Although the PD cases with HHcy in this study were older (in keeping with the fact that older age is associated with HHcy [33]) and had longer disease duration than the PD cases with normal Hcy levels, the differences were not significant. Todorovic *et al* found significantly higher total Hcy levels in LD-treated PD cases with disease duration between 3 and 6 years than in patients with disease duration less than 3 years,[18] a trend that was also noticed in our study.

We acknowledge the limitations of our study including the small sample size, particularly of the treatment naïve PD cases. The cross-sectional design of this study also placed a time limitation so that long term follow up of the different variables from baseline through to final outcome was not possible. Furthermore, we had to rely on patients' account with

respect to drug compliance and dosages used, and this is a possible confounder. Also, other etiologies of HHcy were not evaluated (e.g. folate and vitamin B12 deficiency, and methylene tetrahydrofolate reductase [MTHFR] genotype, and their contributions to HHcy in our cohort cannot thus be determined.

Our study findings lead us to conclude that though HHcy occurs more frequently in PD cases (specifically those on LD in this study), routine measurement of Hcy levels is at present not justifiable considering the cost implications. Prospective studies using larger numbers of PD patients followed longitudinally will further clarify the impact of Hcy on disease outcomes in our PD cases. In the interim, we recommend using vitamin supplementation in PD patients on LD with disease duration greater than three years.

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