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BRIEF ARTICLE

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Important Role of Oxidative Stress Biomarkers in Huntington's Disease

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ABSTRACT: This study examined global oxidative stress (GOS) and antioxidant system and their correlation with disease stage in 19 patients with HD. The results revealed an increase in oxidative stress biomarkers and a reduction in antioxidant systems in HD patients. The effects were more intense in HD1 than in HD2 patients. Additionally, carbonylated proteins and GOS were correlated with disease stage. These findings suggest that oxidative stress plays an important role in the pathogenesis of HD.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant genetic disease characterized by mutant huntingtin protein deposits.[1,2] Different studies have postulated that oxidative stress is involved in the pathogenesis of HD.[3−6] These studies have reported an intense oxidative stress in HD patients compared to healthy subjects.[4−7] Oxidative stress is characterized by an imbalance between reactive oxygen species (ROS) and antioxidant systems. Interestingly, Chen et al.[8] detected a correlation between lipid peroxidation products in plasma and degree of severity in patients with HD and proposed this biomarker as a potential marker for evaluating treatment efficacy. However, existing data are not conclusive.

In view of the foregoing, the main aim of this study was to show the existence of global oxidative stress and antioxidant system deficiency in peripheral samples of patients with HD. All these data were stratified by degree of severity to clarify the role of oxidative stress in Huntington's disease and to evaluate their possible use as biomarkers.

RESULTS

The demographic features of the study groups are presented in Table 1. Table 2 presents the oxidative stress biomarkers obtained in 90% of healthy subjects and the interpretation of oxidative stress markers based on normal cutoff points for our study population of healthy subjects. The correlation between age and oxidative stress biomarkers was also analyzed, but no significant differences were observed (data not shown). In view of these findings, the influence of age on levels of oxidative damage in this clinical study may be excluded.

Changes in Oxidative Stress Biomarkers in HD Patients versus Controls. The results showed that HD patients presented intense oxidative stress characterized by the following:

(1) Oxidative damage. (i) In plasma, HD patients displayed important increases in carbonylated proteins ($P < 0.001$) (Table 3A) and DNA oxidation ($P < 0.01$) (Table 3A). (ii) In erythrocytes, changes were characterized by significant increases in protein and lipid peroxidation ($P < 0.001$ and $P < 0.01$, respectively) (Table 3B).

(2) GSH redox balance. (i) In plasma, HD patients exhibited decreases in reduced glutathione (GSH) content ($P < 0.001$) (Table 3A), increases in oxidized glutathione (GSSG) content ($P < 0.05$) (Table 3A), and decreases in GSH/GSSG ratio ($P < 0.05$) (Table 3A). (ii) In erythrocytes, significant decreases were observed in GSH content ($P < 0.001$) (Table 3B). No significant changes were observed in GSSG and GSH/GSSG ratio (Table 3B).

(3) Antioxidant systems. (i) In plasma, levels of total antioxidant capacity and antioxidant gap were significantly lower in HD patients compared to those in the control group ($P < 0.01$ and $P < 0.05$, respectively) (Table 3A). Moreover, the global oxidative stress marker revealed mild oxidative stress (Figure 2). (ii) In erythrocytes, enzymes showed significant changes characterized by decreases in superoxide dismutase (SOD) ($P < 0.05$) (Table 3B) and increases in glutathione peroxidase (GPx) ($P < 0.001$) (Table 3B).

Changes in Oxidative Stress Biomarkers in Moderate Severity (HD1) or Mild Severity (HD2) versus Controls. Different changes were observed when HD patients were divided according to degree of severity into HD1 (UHDRS > 70) and HD2 (UHDRS < 70) and compared with the control group. Thus, when patients in the HD2 group were compared with controls, significant increases were observed in levels of 8-hydroxy-2-deoxyguonosine (8-OhdG) in plasma ($P < 0.05$) and carbonylated proteins in erythrocytes ($P < 0.05$) (Table 3). In contrast, total antioxidant capacity ($P < 0.05$) (Table 3A) and GSH content decreased in plasma and erythrocytes, respectively ($P < 0.05$) (Table 3B).

Patients in the HD1 group presented significant increases in 8-OHdG levels in plasma ($P < 0.01$) compared to controls

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Table 1. Subject Characteristic and Clinical Ratings of the Huntington's Disease Patients and the Controls[a]

parameter	controls (n = 19)	HD (n = 19)	HD1 (n = 12)	HD2 (n = 7)
gender (male/female)	8/11	9/10	5/7	4/3
age (years)	26−58	24−64	30−64	24−63
height (cm)	162.9 ± 7.4	168.9 ± 8.9	173.1 ± 6.4	157.3 ± 3.5
weight (kg)	78.5 ± 9.19	70.9 ± 8.6	74.5 ± 8.6	64.6 ± 4.1
age at symptom onset (years)		35.1 ± 12.2	31.4 ± 11.5	40.1 ± 11.2
disease duration (years)		11.1 ± 8.1	5.0 ± 3.2	11.9 ± 8.9
expanded CAG repeat no		46.5 ± 4.8	44.6 ± 2.3	48.4 ± 3.9
glucose (mg/dL)	98.9 ± 17.5	91.1 ± 21.4	98.1 ± 25.5	80.5 ± 7.0
cholesterol (mg/dL)	208.5 ± 32.0	191 ± 45.7	192.8 ± 52.2	188.7 ± 41.5
triglycerides (mg/dL)	109.7 ± 32.0	78.0 ± 27.5	74.1 ± 25.8	83.7 ± 32.9
HDL (mg/dL)	57.8 ± 14.5	48.7 ± 12.6	48.0 ± 15.7	49.7 ± 7.8
LDL (mg/dL)	146.8 ± 30.0	126.4 ± 39.8	129.6 ± 41.8	121.5 ± 42.3

[a] Values are expressed as the mean ± SD (range, minimum−maximum).

Table 2

(A) Oxidative Stress Biomarkers in Control Subjects Obtained in 90% of Our Population of Healthy Subjects and Interpretation of Oxidative Stress Markers Based on Normal Cutoff Points According to Our Studied Population of Healthy Subjects[a]

oxidative status	SOD/GPx	total antioxidant capacity (mM)	antioxidant gap (μmol/L)	carbonylated protein (nM)	8-OHdG (ng/mL)
without oxidative stress	≤24.1	≥0.87	≥1012.84	≤0.549	≤68.10
oxidative stress with AEDN	≥24.1	≥0.87	≥1012.84	≥0.549	≥68.10
oxidative stress with AEXD	≤24.1	≤0.87	≤1012.84	≥0.549	≥68.10
oxidative stress with ASGD	≥24.1	≤0.87	≤1012.84	≥0.549	≥68.10

(B) Interpretation of Oxidative Stress Markers in our HD Patients:[b] HD1 (n = 12), HD2 (n = 7) (24)

group	SOD/GPx	total antioxidant capacity (mM)	antioxidant gap (μmol/L)	carbonylated protein (nM)	8-OHdG (ng/mL)
HD (HD1 + HD2)	≤24.1	≤0.87	≤1012.84	≥0.549	≥68.10
HD2	≤24.1	≤0.87	≤1012.84	≤0.549	≤68.10
HD1	≤24.1	≤0.87	≤1012.84	≥0.549	≥68.10

[a] SOD/GPx was analyzed in erythrocytes and the rest of parameters in plasma. AEDN, antioxidant enzymatic deficiency; AEXD, antioxidant extracellular deficiency; ASGD, antioxidant system global deficiency. [b] These data supported the hypothesis of a reduction of AEXD in total HD patients and HD1 patients, whereas the HD2 group shows an important drop in PAO and GAP without increases in PC and 8-OHdG markers, indicating the presence of imbalance in oxidant/antioxidant equilibrium.

(Table 3A). Significant decreases were also observed in total antioxidant capacity ($P < 0.05$) (Table 3A). Protein carbonyls and lipid peroxides in erythrocytes presented significant increases ($P < 0.001$ and $P < 0.05$, respectively), whereas GSH levels and the GSH/GSSG ratio displayed significant decreases ($P < 0.05$ and $P < 0.05$, respectively) (Table 3B).

Changes in Oxidative Stress Biomarkers in HD1 versus HD2. When HD2 and HD1 patients are compared, the differences described above were also observed for an increase in oxidative stress characterized by increases in protein carbonyl content ($P < 0.01$) (Table 3). Along the same lines, positive and statistically significant correlations were only observed between carbonylated proteins and the study groups (control, HD2 and HD1; $r = 0.487$ in plasma and $r = 0.619$ in erythrocytes, $P < 0.01$; Figure 1). Global oxidative stress also increased with disease intensity (controls, 0.33; HD2, 1.75; HD1, 3.03), revealing a significant and positive correlation ($r = 0.851$; $P < 0.01$; Figure 2).

DISCUSSION

This study describes the existence of peripheral oxidative stress in HD patients. The findings also showed that molecular oxidative damage was greater in HD1 patients, coinciding with more severe pathology. These results coincide with those published in scientific literature describing the presence of oxidative stress in HD patients[3,9] and in subjects with other neurodegenerative processes such as Alzheimer's disease, Parkinson's disease, or multiple sclerosis.[5,8,10] In our study, oxidative stress in HD patients was characterized by lipid, protein, and DNA damage; protein carbonylation was more intense in HD1 than in HD2 patients, while no significant differences were observed for the other two markers in HD1 or HD2 patients. These results coincide in part with the findings of other research groups reporting greater oxidative stress in the central nervous system and in plasma in HD patients compared to healthy subjects, as well as between one another depending on process severity.[5,8,10] In line with Klepac et al.,[4] Duran et al.[10] detected higher lipid oxidation levels in symptomatic HD patients than in asymptomatic HD patients, accompanied by increases in levels of lactate (used as a mitochondrial dysfunction marker).

The increases in the oxidation biomarkers analyzed were accompanied by alterations in antioxidant systems. Although the results obtained in this study for glutathione balance coincided

Table 3

(A) Levels of Oxidative Stress Biomarkers in Plasma of Patients with Huntington'S Disease and Control Subjects (Control)[a]

	plasma			
biomarker	control (n = 19)	HD (n = 19)	HD1 (n = 12)	HD2 (n = 7)
carbonylated protein (nM)	0.36 ± 0.14	0.571 ± 0.24[b]	0.6 ± 0.25	0.49 ± 0.22[e]
8-OHdG (ng/mL)	45.51 ± 11.93	69.53 ± 32.23[c]	71.5 ± 24.0	65.70 ± 26.97
PAO (mM)	1.07 ± 0.20	0.83 ± 0.08[c]	0.83 ± 0.085	0.82 ± 0.08
GAP (mM)	1283.7 ± 374.5	1017.5 ± 200.97[d]	996.2 ± 216.1	1051.4 ± 192.4
GSH (μM)	30.19 ± 4.84	24.63 ± 7.44[b]	24.03 ± 8.47	24.68 ± 5.27
GSSG (μM)	9.95 ± 5.9	13.06 ± 8.11[d]	13.73 ± 9.32	11.59 ± 5.06
GT	42.15 ± 6.52	37.25 ± 7.40	37.71 ± 9.42	36.24 ± 3.18
ratio	2.98 ± 1.75	1.73 ± 0.97[d]	1.55 ± 1.04	2.10 ± 0.83

(B) Levels of Oxidative Stress Biomarkers in Erythrocytes of Patients with Huntington's Disease and Control Subjects (Control)[a]

	erythrocytes			
biomarker	control	HD	HD1	HD2
carbonylated protein (nM/g Hb)	0.46 ± 0.04	0.66 ± 0.19[b]	0.71 ± 0.45	0.57 ± 0.15[f]
LPO (μM/g Hb)	11.68 ± 3.73	15.75 ± 5.83[c]	16.17 ± 5.8	14.82 ± 3.10
GSH (μM/g Hb)	141.5 ± 30.6	122.8 ± 14.87[b]	126.2 ± 12.26	114.72 ± 18.8
GSSG (μM/g Hb)	42.47 ± 29.3	44.95 ± 15.29	46.19 ± 16.27	40.58 ± 15.74
GT	184.1 ± 28.3	167.82 ± 18.2	172.45 ± 17.3	155.36 ± 16.2
ratio	3.09 ± 1.62	2.73 ± 1.04	2.73 ± 1.04	2.82 ± 1.25
SOD ((U/mL)/g Hb)	2.24 ± 0.18	1.64 ± 0.21[d]	1.29 ± 0.74	1.86 ± 0.78
GPx ((U/mL)/g Hb)	0.20 ± 0.1	0.60 ± 0.14[b]	0.6 ± 0.01	0.5 ± 0.017

[a] Expresses as the mean ± SD. [b] $P < 0.001$ vs control. [c] $P < 0.01$ vs control. [d] $P < 0.05$ vs control. [e] $P < 0.01$ vs HD1. [f] $P < 0.05$ vs HD1.

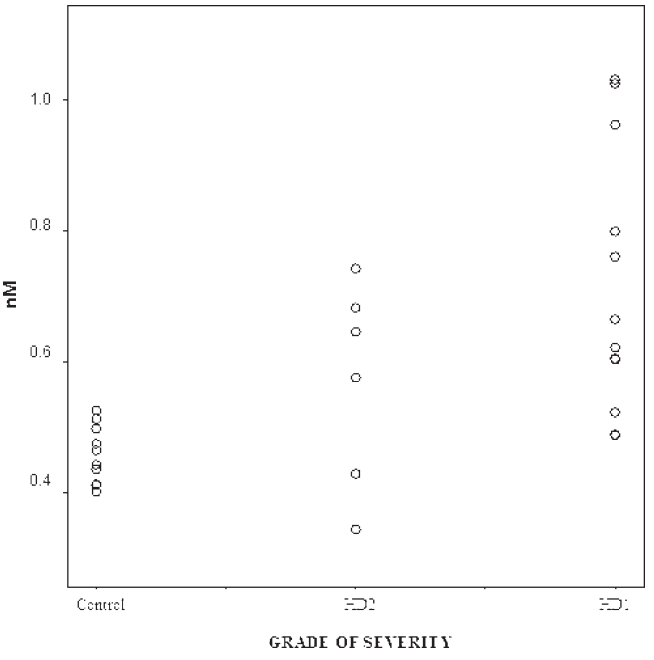

Figure 1. Correlation between levels of carbonylated proteins in erythrocytes and disease stage in patients with HD (HD1 and HD2) and healthy subject (controls). HD1 score is UHDRS > 70. HD2 score is UHDRS < 70. $r = 0.619$; $P < 0.01$.

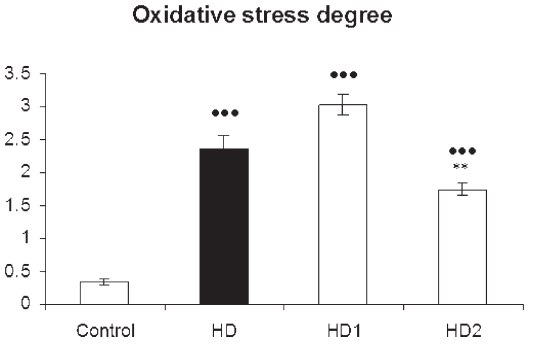

Figure 2. Oxidative stress degree in patients with different stage of disease, showing significant and positive correlation ($r = 0.851$; $P < 0.01$). Bar indicates ±SD. HD1 score is UHDRS > 70. HD2 score is UHDRS < 70. ●●●, $P < 0.001$ vs control. **, $P < 0.05$ vs HD1.

with those reported elsewhere,[4] contradictory and controversial results were obtained for antioxidant enzyme activity; some authors have reported decreases in SOD and GPx antioxidant enzyme activity,[8,10−13] while others have observed increases.[5,14−16] This may be at least partly due to (i) the different degrees of severity displayed by the patients studied, (ii) oxidative damage intensity, (iii) the medium analyzed and methodology used, (iv) the quantification protocols used, and/or (v) the sample handling techniques employed.[4,5]

Changes in oxidative stress biomarkers are normally analyzed and evaluated on an isolated and static basis, and the existence of oxidative stress is interpreted as an increase in oxidative damage indicators and/or a decrease in antioxidant systems. This study therefore aimed to examine and dynamically analyze general oxidative balance, evaluating overall oxidative status.[17,18] The

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ratio between SOD/GPx[19] enzymes in HD patients decreased in line with normal levels. Such decreases were observed in HD1 and HD2 patients; no statistically significant changes were observed between both groups. These results would initially suggest that antioxidant enzyme system capacity remained intact in the patients studied, probably indicating that these systems were functioning properly despite the presence of oxidative stress.

The study of changes in antioxidant gap revealed a decrease in this marker in HD patients with respect to the controls. This supported the hypothesis that residual plasmatic antioxidant capacity had decreased significantly. These findings reveal two possible scenarios: (i) a deficiency in molecules such as ascorbic acid, α-tocopherol, and other minority elements and (ii) the importance of these molecules with antioxidant capacity in oxidative stress development and intensity in HD patients. These results coincide with those reported by Beristain-Pérez et al.[18] in processes such as diabetes mellitus, high blood pressure, and osteoarthritis, characterized by decreases in antioxidant gap and their important role in triggered oxidative symptoms and in the evolution of the clinical process, indirectly confirming the results described here and the aforementioned hypothesis.

On the basis of these results, global oxidative stress was evaluated in the patients studied using the construct established by Beristain-Pérez et al.[18] This construct showed that HD patients presented higher scores than the controls, suggesting the presence of global oxidative stress in these patients. Global oxidative stress levels were also higher in HD1 than in HD2 patients, associated with the intensity of clinical symptoms.[4]

HD patients displayed alterations in antioxidant gap, total antioxidant capacity, carbonylated proteins, and oxidized DNA, suggesting increased production of intracellular ROS, causing ROS to increase and accumulate. These radicals would have an oxidative effect at the intra- and extracellular levels. At the intracellular level, they would trigger DNA, intracellular protein, and membrane lipid oxidation. Their diffusion into the extracellular medium would induce extracellular protein and lipid oxidation, generally resulting in increased levels of lipid peroxidation products, 8-OHdG and carbonylated proteins, accompanied by a relevant and significant decrease in the antioxidant gap, i.e., in minority plasmatic antioxidants (vitamin C, α-tocopherol, etc.). All these phenomena coincide with the correct functioning of the primary axis of antioxidant enzyme systems (evaluated as SOD/GPx) and the maintenance of total antioxidant capacity. All these results would support the hypothetical decrease in exogenous antioxidant capacity. It may also be postulated that this phenomenon is progressive because the HD2 patients displayed significant reductions in total antioxidant capacity and antioxidant gap, whereas carbonylated proteins and 8-OHdG levels did not exceed normal cutoff points. Nevertheless, HD1 patients displayed increases in carbonylated proteins and 8-OHdG, accompanied by decreases in total antioxidant capacity and antioxidant gap.

Finally, a close correlation was observed between carbonylated proteins and disease severity and between disease severity and global oxidative stress. These correlations, together with the statistically significant changes in the other oxidative stress variables analyzed (GSH, malondialdehyde (MDA), etc.), would indicate that carbonylated proteins and global oxidative stress are the two variables most sensitive to oxidative changes associated with the evolution of HD. These findings could support their clinical use as markers to monitor the evolution of the disease.

CONCLUSIONS

It may be concluded that (i) HD patients presented a global oxidation status, (ii) global oxidative stress was more intense in HD1 than in HD2 patients, (iii) HD patients exhibited decreases in nonenzymatic extracellular antioxidant systems, and (iv) carbonylated proteins and global oxidative stress may be two useful biochemical oxidative stress markers for monitoring the evolution of HD patients. Nevertheless, further research is necessary in this area to clarify and resolve all doubts regarding the relevance and diagnostic−clinical use of individual and combined (constructs of antioxidant gap and global oxidative stress) oxidative stress biomarkers in HD.

MATERIALS AND METHODS

Patients. The study was conducted with 19 patients from the Departments of Neurology at Reina Sofia University Hospital in Cordoba (13) and Valme Hospital in Seville (6). Their diagnoses were confirmed genetically by the Department of Genetics at both hospitals. The patients were divided into two groups based on disease severity rated according to the Unified Huntington's Disease Rating Scale (UHDRS):[20] (i) HD1 (UHDRS > 70); (ii) HD2 (UHDRS < 70). Nineteen healthy subjects (controls at age 42.23 ± 9.24; 8 men/11 women) were recruited, matched according to age and sex. Inclusion and exclusion criteria were fulfilled by 19 patients (age 43.50 ± 10.62; 9 men/10 women). Inclusion criteria were the following: informed consent; CAG trinucleotide expansion (35−49 repeats); age, 24−64 years; >25 score in mini mental state examination (MMSE);[21] age at symptom onset, 31.42 ± 11.51 years; disease duration, 11.18 ± 8.10 years; total cholesterol concentration, 191.0 ± 45.7 mg/dL; triglyceride concentration, 78.0 ± 27.5 mg/dL; HDL concentration, 48.7 ± 12.6 mg/dL; LDL concentration, 126.4 ± 39.8 mg/dL. Exclusion criteria were the following: chronic inflammatory disease, infectious illness 3 days before beginning the study, diabetes mellitus, chronic heart disease, anemia, vitamin or antioxidant supplement intake, smoking, drinking (alcohol and exciting).

Extraction was performed between 09:00 and 10:00 a.m. Blood samples were obtained from the antecubital vein and collected in tubes containing 1 mg/mL EDTA-K_3 as anticoagulant, after 12 h of fasting. Immediately after, plasma was separated from red cells by centrifugation at 2500 rpm at 4 °C for 15 min and the fraction was frozen in aliquots and stored at −85 °C for later study.

Biochemical Parameters. The amount of carbonylated proteins in plasma and erythrocytes was evaluated using the method described by Levine et al.[22] Lipid peroxidation products (MDA + 4-HDA) were estimated in erythrocytes using the method described by Eldermeier et al.[23] Oxidative DNA adduct 8OHdG (8-OHdG Check-437-01222) and total antioxidant capacity (PAO, KPA-050) were assayed in plasma using kits purchased from JaICA (Japanese Institute for the Control of Aging, Fukuroi City, Shizuoka, Japan). Total glutathione (GSSG + GSH) and GSH levels in plasma and erythrocytes were evaluated using the Bioxytech GSH-420 and GSH-400 kits, respectively (Oxis International, Portland, OR, U.S.). GSSG levels were calculated by subtracting GSH from total glutathione, and the GSH/GSSG ratio was determined. Antioxidant enzyme activity was measured in red blood cells: GPx activity was evaluated by the Flohé and Gunzler method (1984), and SOD activity was evaluated using a colorimetric assay kit purchased from BioVision (Mountain View, CA, U.S.). The SOD/GPx ratio was calculated as the correct functioning of the main axis of enzymatic antioxidant systems. This ratio indicates antioxidant enzyme system efficiency, and its evaluation is more important than absolute enzymatic activity.

The antioxidant gap was calculated using the following equation: gap = total antioxidant capacity − ([albumin × TEAC] + [uric acid × TEAC]). This indicator consists of evaluating the antioxidant activity

in plasma of ascorbic acid, α-tocopherol, bilirubin, transferrin, and other minority antioxidant compounds, excluding albumin, uric acid, and Trolox equivalent. Sánchez-Rodriguez et al.[18] designed an integral method for analyzing oxidative damage that includes different oxidative stress and antioxidant system parameters: SOD/GPx ratio, total antioxidant capacity, antioxidant gap, and two oxidative damage biomarkers (lipid peroxidation products and 8-OHdG). The same design was used in this study, although lipid peroxidation products were substituted with carbonylated proteins because these are more sensitive oxidative stress markers. These data may produce four possible scenarios: (i) no oxidative stress, (ii) antioxidant enzyme deficiency (AEDN), (iii) antioxidant extracellular deficiency (AEXD), and (iv) antioxidant system global deficiency (ASGD) (Table 2).

The modified construct described by Berinstain-Perez et al.[18] was used to calculate global oxidative stress. This construct incorporated seven parameters: (i) two oxidation damage markers (lipid and DNA oxidation in plasma) (protein oxidation in plasma was used instead of lipid); (ii) three enzymatic antioxidant biomarkers in erythrocytes (SOD, GPx, and SOD/GPx ratio); (iii) two nonenzymatic antioxidant markers in plasma (total antioxidant capacity and antioxidant gap). A value 0 or 1 was assigned to each parameter depending on whether it was normal or not normal, respectively. Normality was defined as the value including 90% of controls for each variable. Thus, the score was established by adding all items, with a maximum of 7 points and a minimum of 0 points: no oxidative stress, ≤1 point; mild oxidative stress, ≤3 points; moderate oxidative stress, ≤4 points; severe oxidative stress, ≤6 points.

Statistical Analysis. Statistical evaluation was carried out using the SPSS17.0 statistical software package (SPSS Iberica, Madrid, Spain) for Windows. The Shapiro−Wilk test did not show significant departures from normality in the distribution of variance values. Statistical significance was measured using the one-way analysis of variance (one-way ANOVA), corrected with the Bonferroni test. Pearson's correlation analysis was used to evaluate the correlation between variables. $P < 0.05$ was considered significant. Data are presented as the mean ± SD. Normal values were determined with the base of 90% of healthy subjects.

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ABBREVIATIONS USED

AEDN, antioxidant enzymatic deficiency; AEXD, antioxidant extracellular deficiency; ASGD, antioxidant system global deficiency; GSH, reduced glutathione; GSSG, oxidized glutathione; GPx, glutathione peroxidase; HD, Huntington's disease; HD1, Huntington's disease with moderate severity; HD2, Huntington's disease with mild severity; MDA, malondialdehyde; MMSE, mini mental state examination; 8-OHdG, 8-hydroxy-2′deoxyguanosine; ROS, reactive oxygen species; SOD, superoxide dismutase; TEAC, Trolox equivalent antioxidant capacity; UHDRS, Unified Huntington's Disease Rating Scale

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