Assessment of DNA Damage in the Peripheral Lymphocytes of Patients with Parkinson's Disease and Multiple Sclerosis Using the Comet Assay

Parkinson ve Multipl Skleroz Hastalarının Periferal Lenfositlerindeki DNA Hasarının Comet Yöntemiyle Değerlendirilmesi

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ABSTRACT Objective: It has been suggested that oxidative damage have been involved in the pathogenesis of neurodegenerative diseases. Although the role of oxidative stress in the etiology of these diseases is not clear, the increased levels of DNA damage and protein as well as DNA and mitochondrial oxidation have been demonstrated in patients with Parkinson's disease (PD) and Alzheimer's disease (AD) in a few studies. **Material and Methods:** In this study, the levels of DNA damage in the peripheral lymphocytes of patients with PD and multiple sclerosis (MS), and their age-matched healthy controls were determined by the comet assay with and without treatment of blood samples with 50μM hydrogen peroxide (H2O2) and the oxidative purine specific DNA repair enzyme, formamidopyrimidine glycosylase (Fpg). **Results:** The DNA damage in the lymphocytes of the patients with PD has been significantly increased compared to controls. However, no significant increase in the DNA strand breakage was detected in the patients with PD after treatment with either H2O2 or Fpg. Although the DNA damage in the MS patients was higher than their healthy controls, the difference was not found to be significant. A significant increase observed in the DNA damage after treatment with H2O2 and with Fpg in MS patients, have suggested the susceptibility to oxidative DNA damage in these patients. **Conclusion:** The data presented in this paper shows high level of DNA damage in the lymphocytes of patients with PD and MS which indicates the possibility of oxidative stres in these neurodegenerative diseases.

Key Words: Neurodegenerative diseases; multiple sclerosis; Parkinson disease; DNA damage; comet assay

ÖZET Amaç: Sinir dokusunun bozulumu ile ilgili hastalıkların gelişiminde oksidatif hasarın katkısı olduğu öne sürülmektedir. Bu hastalıkların etiyolojisinde oksidatif baskının rolü açık olmamakla birlikte, Parkinson hastalarında (PH) ve Alzheimer hastalarında (AH) DNA hasarının ve protein, DNA ve mitokondri oksidasyonunun arttığını gösteren az sayıda çalışma bulunmaktadır. **Gereç ve Yöntemler:** Bu çalışmada, Parkinson ve Multipl Skleroz (MS) hastalarının ve yaşları eşleştirilmiş sağlıklı kontrollerinin periferal lenfositlerindeki DNA hasar düzeyi, kan örneklerine 50 μM hidrojen peroksit (H_2O_2) ve oksidatif pürin özgül DNA onarım enzimi, formamidopirimidin glikozilaz (Fpg) uygulamasından önce ve sonrasında Comet yöntemiyle saptanmıştır. **Bulgular:** Parkinson hastalarının lenfositlerinde DNA hasarı kontrollere kıyasla önemli şekilde artmıştır. Bununla birlikte, Parkinson hastalarının lenfositlerindeki DNA iplik kırıklarında H_2O_2 ya da Fpg uygulaması ile önemli artış meydana gelmemiştir. MS hastalarının DNA hasarı sağlıklı kontrollerininkine kıyasla yüksekken, aradaki fark istatistiksel olarak anlamlı bulunmamıştır. MS hastalarının lenfositlerindeki DNA hasarında H_2O_2 ve Fpg uygulamasının ardından önemli artış gözlenmiştir, bu hastaların oksidatif DNA hasarına duyarlı oldukları ileri sürülmüştür. **Sonuç:** Bu makalede sunulan sonuçlar, Parkinson ve MS hastalarının lenfositlerindeki yüksek düzey DNA hasarını ve aynı zamanda sinir dokusunun bozulumu ile ilgili hastalıklarda oksidatif baskı olasılığını göstermektedir.

Anahtar Kelimeler: Nörodejeneratif hastalıklar; multipl skleroz; Parkinson hastalığı; DNA hasarı; comet assay

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Neurodegenerative diseases are a group of nervous system disorders that are characterized by progressive death of various neurons to impair the motor functions, memory, emotional conditions, and learning. The group includes Alzheimer's disease (AD), amyotrophic lateral sclerosis, spinal muscular atrophy, myotonic dystrophy, Parkinson's disease (PD), and Multiple Sclerosis (MS), etc. The etiology of most neurodegenerative disorders is multifactorial and consists of an interaction between environmental factors and genetic predisposition.[1-4] Oxidative stress resulting from an imbalance between the formation of free radicals and the antioxidant defense systems, may be a potential pathophysiological factor involved in the degenerative processes. Although the role played by oxidative stress in neurodegenerative diseases remains undefined, some previous studies mostly on AD have provided evidence of the involvement of oxidative damage in the pathogenesis of neurodegenerative diseases.[5-10] Since brain has many metabolic activities, high energy requirements and a high oxygen consumption rate, it can be exposed to reactive oxygen species deeply. It is estimated that oxidative stress can be an important factor in degenerative neural death and disease progression. Increased levels of redox active iron and lipid peroxidation, increased RNA oxidation and nuclear DNA oxidation, increased mitochondrial oxidation, decreased level of neural polyunsaturated fatty acid and protein oxidation have been demonstrated in patients with AD.[11-16]

A significantly higher levels of basal and oxidative DNA damage were found in the lymphocytes as well as in the polymorphonuclear leukocytes of patients with AD and with mild cognitive impairment (MCI).[17-19] Studies in patients with AD showed increased levels of 8-hydroxyguanosine (8OHG) or 8-hydroxy-2'-deoxyguanosine (8OHdG) in addition to decreased levels of plasma antioxidants as compared to controls.[5,11,17,20-23] On the other hand, most studies on patients with AD have been performed in post-mortem tissues with advanced disease without clarifying whether oxidative stress is an early event or a common final step secondary to the degenerative process. Only relatively small number of studies have been performed in living patients with AD suggesting that oxidative stress in AD is detectable not only in the central nervous system, but also in peripheral cells.[11,17-19,24]

PD which is characterized by a severe neurodegeneration of the mesencephalic dopaminergic neurons in the substantia nigra, is one of the most common neurodegenerative diseases affecting about 2% of the population over 60 years.[25] It causes extrapyramidal motor dysfunction as tremor, rigidity, and dyskinesia. The pathogenic mechanism of the disease is not clear and oxidative stress seems to be one of the main etiological factors. Protein oxidation has been demonstrated to be elevated in Lewy bodies in cases of PD. There are also a few studies indicating the elevated levels of oxidative DNA damage in the lymphocytes of patients with PD.[26-29]

MS is a common, often disabling disease of the central nervous system. Although it is generally accepted that vascular factors, metabolic alterations, viral infections of the central nervous system and disturbed immune mechanisms are responsible, little is known about its cause or the factors that contribute to its unpredictable course. [30,31]

In the present study, in order to investigate the possible role of oxidative stress in neurodegerative diseases, the levels of DNA damage, susceptibility to oxidative DNA damage in peripheral lymphocytes of patients with MS and PD and their comparable age-matched healthy controls were determined by single cell gel electrophoresis or comet technique which has been in use for many years as a sensitive method to detect DNA single strand breaks, and alkaline-labile sites, DNA-DNA or DNA-protein crosslinking and also oxidative DNA adducts and SSBs associated with incomplete excision repair sites in single cells.[32]

MATERIAL AND METHODS

SUBJECTS

The study consisted of 25 patients with PD (7 females and 18 males, mean age of 64 ± 2.8 years and age range of 31-86 years) and 22 MS patients (16

females and 6 males, with a mean age of 36 ± 2.6 years and age range of 22-66 years) who were the outpatients of the Numune Education and Research Hospital, 2nd Neurology Clinic, Ankara Turkey. For PD patients, 14 healthy subjects (12 females and 2 males, with a mean age of 59 ± 4.5 years, age range of 30-81 years), and for MS patients, 16 healthy subjects (13 females and 3 males, with the mean age of 40 ± 3.6 years and age range of 19-68 years) of comparable age, socioeconomic life-style and smoking habits were chosen as controls. The study has been approved by the local ethics committee according to the 'Decleration of Helsinki'. The clinical diagnosis of PD was based on the presence of the symptoms of postural instability according to UK Parkinson's Disease Society Bank Criteria whereas MS patients were diagnosed according to McDonald's Criteria. Eighteen PD patients had been treated with levodopa plus dopa-dicarboxylase (DDC) inhibitors such as benserazide and carbidopa for more than 5 years and the remaining 7 patients had been treated with other drugs such as galantamine, pramipexole. Fourteen MS patients were not using drug therapy at the time of the study whereas 8 MS patients had been treated with interferon. Standardized health questionnaires from all patients and controls relating to their occupational exposure and life-style factors such as smoking, drug consumption, viral diseases, radiodiagnostic examinations and dietary habits were obtained. None of the control subjects had verifiable symptoms of dementia or other internal and neurological disorders, as judged by their clinical examination in which results of routine physical, neurological and neuropsychological tests were within the normal values. None of the subjects had also been taking antioxidants such as vitamin E or any kind of medication known to interfere with the redox state of the body or to cause oxidative or DNA damage. All participating subjects were provided with a written informed consent before their blood samples were drawn.

BLOOD SAMPLES AND CELL PREPARATION

2 ml heparinized whole blood was collected by venipuncture from the patients and the controls. Lymphocytes were isolated immediately by Ficoll-Hypaque density gradient and washed with PBS.[33] An aliquot of cells was used to check for viability by trypan blue exclusion.

SLIDE PREPARATION

The basic alkaline technique of Singh et al., described by Collins et al. was followed.[34,35] All microscopic slides had been covered with 1% NMA at about 45 ºC in Ca^{2+} - and Mg^{2+}- free PBS before the experiment. This layer was used to promote the attachment of the second layer. For the second layer, around 10000 cells mixed with 80 µl of 1% LMA (pH 7.4) were rapidly pipetted onto this slide, spread using a cover slip, and maintained on an ice-cold flat tray for 5 min to solidify. After removal of the cover slip, the slides were immersed in cold, freshly prepared lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, pH 10) with 1% Triton X-100 and 10% DMSO added just before use, for a minimum of 1 h at 4 ºC to remove cellular proteins. Each analysis was done in duplicate and carried out immediately after sample collection without freezing or storing. For Fpg-modified comet assay; after lysis and slides having H_2O_2 treated cells were washed three times for 5 min in enzyme buffer (20mM Tris HCl, 1mM Na_2EDTA, 100 mM NaCl, 0.5mg BSA/ml, pH 7.0). Then, 6 µl agarose MEEO was placed at the corners of a coverslip and slides were incubated with 200 µl of Fpg in enzyme buffer solution. Control slides were treated with 200µl enzyme buffer only. Slides were placed horizontally in humidity chamber at 37 ^{0}C for 30 min. DNA unwinding and electrophoresis were then completed as described below.

ELECTROPHORESIS

The slides were removed from the lysing solution, drained and placed in horizontal gel electrophoresis tank side by side, avoiding spaces and with the agarose ends facing each other, nearest the anode. The tank was filled with fresh electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. Before electrophoresis, the slides were left in the solution for 20 min at 4^{0}C to allow the unwinding of the DNA and expression of alkaline la-

bile damage. Electrophoresis was conducted at a low temperature (4 ºC) for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level and using a compact power supply (Power Pack P 25 Biometra Analytic GmbH). All of these steps were conducted under dimmed light to prevent the occurrence of an additional damage. After electrophoresis, the slides were taken out of the tank, washed in distilled water. Tris buffer (0.4 M Tris, pH 7.5) was added drop-wise and gently to neutralize the excess alkali and the slides were allowed to sit for 5 min. The neutralizing procedure was repeated 3 times. After waiting the slides each for 5 min in distilled water, 50%, 75% and 99% ethanol they were allowed to dry at room temperature.

STAINING AND SLIDE SCORING

To each slide, 30 µl of EtBr (20 µl/ml) was added. For visualization of DNA damage, slides were examined at a 1000X magnification using a 40X objective on a fluorescence microscope Leica (Wetzlar, Germany). Measurements were made by a computer-based image analysis system 'Comet Assay III' Perceptive Instruments (Suffolk, England). Images of 100 randomly selected lymphocytes, i.e. 50 cells from each of two replicate slides were analyzed from each sample and tail migration, tail moment and tail intensity were measured. Breaks in the DNA molecule disturb its complex supercoiling, allowing liberated DNA to migrate towards the anode. Staining shows the DNA as 'comets'. The mean value of the tail migration, tail moment and tail intensity was calculated and used for the evaluation of DNA damage.

STATISTICAL ANALYSIS

The SPSS 11.5 for Windows computer program was used for statistical analysis. Statistical comparison of the results from controls and patients with PD and MS, were carried out by Mann-Whitney U test. Intragroup comparisons for the differences between peripheral lymphocytes, H_2O_2-treated peripheral lymphocytes, and FPG+H_2O_2-treated peripheral lymphocytes were evaluated by Wilcoxon Sign Rank tests. The percent change values of H_2O_2-treated peripheral lymphocytes and Fpg+H_2O_2-treated peripheral lymphocytes were calculated according to peripheral lymphocytes and H_2O_2-treated peripheral lymphocytes, respectively. Results were expressed as median (minimum-maximum). $p<0.05$ was considered statistically significant.

RESULTS

Cell viability, as tested using trypan blue dye exclusion of each treated group, was more than 90%. The DNA damage expressed as tail migration, tail moment and tail intensity in the lymphocytes of the patients with PD and MS and also their healthy controls are given in tables (Table 1-3). The DNA damage was found to be significantly higher in patients with PD compared to their healthy controls ($p<0.05$). However, no significant increase in DNA strand breakage was found after treatment with only H_2O_2 and with H_2O_2 and Fpg in the lymphocytes of patients with PD compared to their healthy controls (Figure 1).

Although the basal DNA damage was higher in MS patients compared to their healthy controls, the difference was not statistically significant. Although no significant difference was found in the tail migration and tail intensity of the patients and controls, a significant increase in the tail moment after treatment of lymphocytes with H_2O_2 and post-treatment with Fpg have been observed in the patients with MS compared to their healthy controls (Figure 2).

DISCUSSION

The brain is only 2-3% of the total body mass, but it consumes 20% of the body oxygen. Cells in the brain are particularly susceptible to oxidative damage due to the high levels of polyunsaturated fatty acids in their membranes and relatively low activity of endogenous antioxidant enzymes. On the other hand, aging is also associated with increased oxidative stress and accumulation of oxidatively damaged biomolecules which gradually weakens cognition. The increase in oxidative stress together with the decline in endogenous antioxidants are important underlying risk factors for older people to develop neurodegenerative diseases.[36-38] More

TABLE 1: Assessment of DNA damage as expressed tail migration in peripheral blood lymphocytes of patients with Parkinson's disease (PD) and multiple sclerosis (MS) and their healthy controls.[a]

	(A) PD patients (n= 25)	(B) Controls of PD patients (n=14)	p	(C) MS patients (n=22)	(D) Controls of MS patients (n=16)	p
(E) Peripheral lymphocytes	19.61 (13.23;41.03)	16.76 (14.78;20.67)	0.016	17.35 (14.13;31.64)	17.67 (13.90;21.77)	0.827
(F) H_2O_2 Treated peripheral	27.53 (17.53;49.90)	30.23 (16.91;46.8)	0.761	28.27 (9.73;43.44)	22.27 (16.91;39.18)	0.084
Lymphocytes	0.36 (-0.33;2.77)[b]	0.59 (-0.05;2.04)[b]		0.53 (-0.31;1.80)[b]	0.22 (-0.05;1.54)[b]	
(G) Fpg+H_2O_2 treated	39.18 (24.63;59.32)	39.97 (30.89;50.53)	0.965	44.93 (11.33;78.99)	38.80 (30.89;49.22)	0.162
Peripheral lymphocytes	0.38 (-0.38;2.06)[c]	0.33 (-0.21;1.84)[c]		0.54 (-0.10;2.42)[c]	0.63 (-0.21;1.84)[c]	
Within comparison						
(E)-(F)	0.001	0.002		<0.001	0.001	
(F)-(G)	<0.001	0.004		<0.001	0.001	

[a]The results are given as median (minimum-maximum).
[b]Second row gives the summarize of percent change values according to peripheral lymphocytes.
[c]Second row gives the summarize of percent change values according to H_2O_2 treated peripheral lymphocytes.

TABLE 2: Assessment of DNA damage as expressed tail moment in peripheral blood lymphocytes of patients with Parkinson's disease (PD) and multiple sclerosis (MS) and their healthy controls.[a]

	(A) PD patients (n= 25)	(B) Controls of PD patients (n=14)	p	(C) MS patients (n=22)	(D) Controls of MS patients (n=16)	p
(E) Peripheral lymphocytes	0.89 (0.29;8.82)	0.67 (0.22;1.13)	0.008	0.62 (0.17;3.79)	0.61 (0.29;3.62)	0.988
(F) H_2O_2 Treated peripheral	3.65 (0.30;11.56)	5.05 (0.56;13.15)	0.740	4.20 (0.55;18.07)	1.98 (0.56;6.33)	0.024
Lymphocytes	0.60 (-0.68;38.86)[b]	6.48 (-0.40;25.59)[b]		3.71 (-0.05;38.76)[b]	1.44 (-0.40;9.38)[b]	
(G) Fpg+H_2O_2 treated	6.46 (1.30;15.87)	6.05 (3.14;12.83)	0.942	8.09 (1.91;14.74)	5.19 (3.14;10.52)	0.009
peripheral lymphocytes	0.91 (-0.34;11.84)[c]	0.41 (-0.47;17.79)[c]		0.90 (-0.51;6.48)[c]	0.90 (-0.47;17.79)[c]	
Within comparison						
(E)-(F)	0.003	0.002		<0.001	0.001	
(F)-(G)	<0.001	0.019		0.006	0.002	

[a]The results are given as median (minimum-maximum).
[b]Second row gives the summarize of percent change values according to peripheral lymphocytes.
[c]Second row gives the summarize of percent change values according to H_2O_2 treated peripheral lymphocytes.

TABLE 3: Assessment of DNA damage as expressed tail intensity in peripheral blood lymphocytes of patients with Parkinson's disease (PD) and multiple sclerosis (MS) and their healthy controls.[a]

	(A) PD patients (n= 25)	(B) Controls of PD patients (n=14)	p	(C) MS patients (n=22)	(D) Controls of MS patients (n=16)	p
(E) Peripheral lymphocytes	6.24 (2.64;32.56)	4.47 (1.59;7.29)	0.012	5.01 (1.60;18.79)	4.50 (2.08;14.47)	0.510
(F) H_2O_2 Treated peripheral	16.37 (2.11;39.28)	21.03 (5.00;41.48)	0.654	18.96 (0.03;39.83)	11.26 (5.00;24.92)	0.078
Lymphocytes	0.22 (-0.64;11.22)[b]	3.41 (-0.12;13.92)[b]		1.86 (-1.00;15.84)[b]	1.30 (-0.12;4.36)[b]	
(G) Fpg+H_2O_2 Treated	26.16 (8.77;45.48)	23.69 (13.03;44.30)	0.874	28.77 (0.25;48.79)	21.71 (13.03;40.53)	0.201
Peripheral lymphocytes	0.69 (-0.12;6.38)[c]	0.40 (-0.43;7.11)[c]		0.72 (-0.99;3.04)[c]	0.62 (-0.43;7.11)[c]	
Within comparison						
(E)-(F)	0.005	0.001		<0.001	0.001	
(F)-(G)	<0.001	0.019		0.007	0.003	

[a]The results are given as median (minimum-maximum).
[b]Second row gives the summarize of percent change values according to peripheral lymphocytes.
[c]Second row gives the summarize of percent change values according to H_2O_2 treated peripheral lymphocytes.

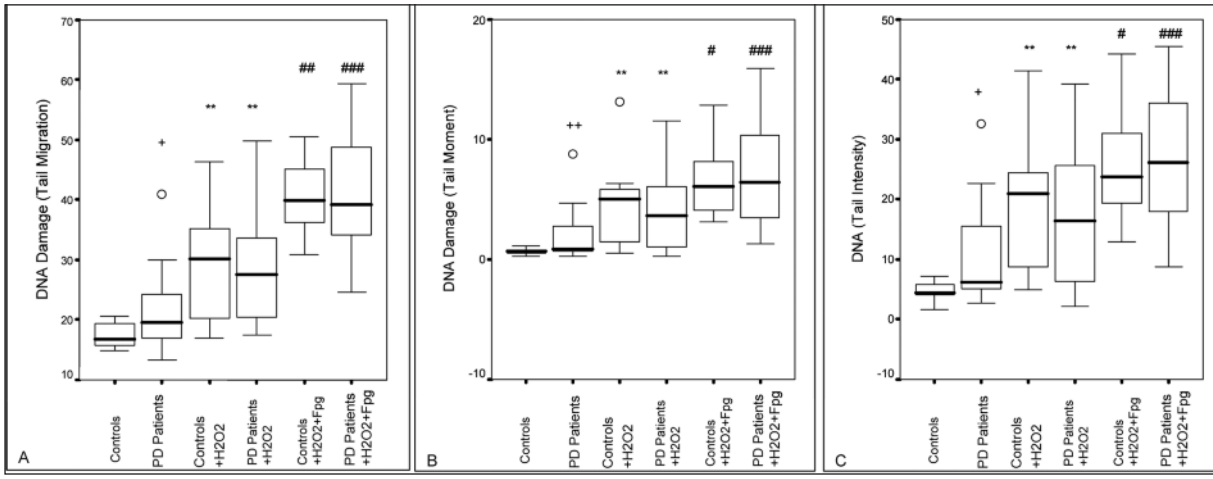

FIGURE 1: Assessment of DNA damage in peripheral blood lymphocytes of patients with Parkinson's disease (PD) and healthy controls.
DNA damage in peripheral lymphocytes of the groups expressed as **(A)** tail migration, **(B)** tail moment, and **(C)** tail intensity.
**p<0.01, DNA damage in peripheral lymphocytes of patients with Parkinson's disease and healthy controls compared to their H_2O_2 treated samples.
#p<0.05; ##p<0.01, ###p<0.001, DNA damage in H_2O_2 treated peripheral lymphocytes of patients with Parkinson's disease and healthy controls compared to their H_2O_2+Fpg treated samples.
+p<0.05; ++p<0.01, DNA damage in peripheral lymphocytes of patients with Parkinson's disease compared to healthy controls.

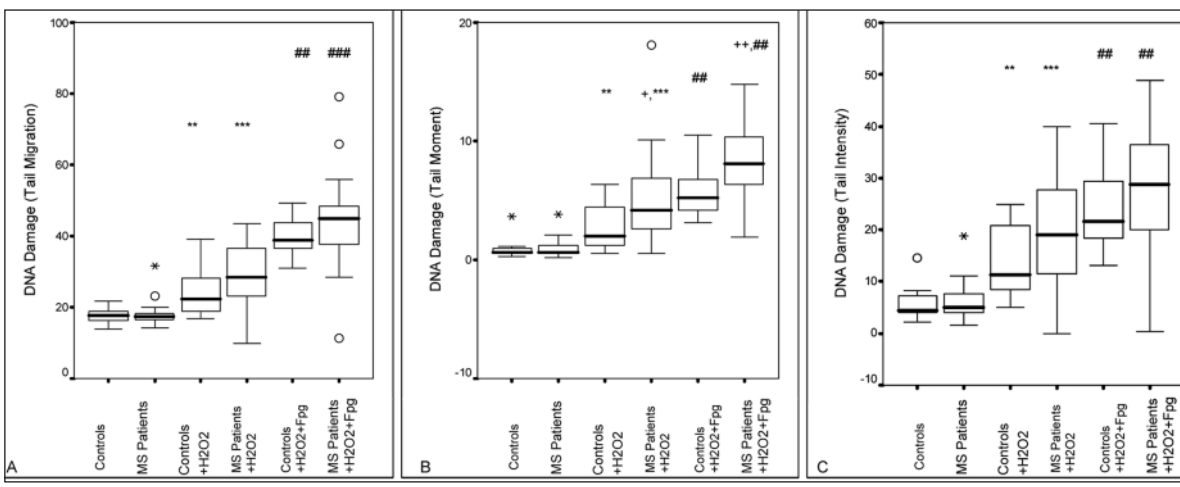

FIGURE 2: Assessment of DNA damage in peripheral blood lymphocytes of patients with multiple sclerosis disease (MS) and healthy controls.
DNA damage in peripheral lymphocytes of the groups expressed as **(A)** tail migration, **(B)** tail moment, and **(C)** tail intensity.
** p<0.01; *** p<0.001, DNA damage in peripheral lymphocytes of patients with MS and healthy controls compared to their H_2O_2 treated samples.
p<0.01, ### p<0.001, DNA damage in H_2O_2 treated peripheral lymphocytes of patients with MS and healthy controls compared to their H_2O_2+Fpg treated samples.
+ p< 0.05; ++ p<0.01, DNA damage in peripheral lymphocytes of patients with MS compared to healthy controls.

oxidized proteins such as carboxyls and nitro-protein adducts were detected in both nuclear and mitochondrial DNA in the elderly brain. Post mortem studies demonstrated increased oxidation of proteins, lipids and DNA in neurodegenerative diseases including AH, PD, stroke, amyotrophic lateral sclerosis, etc.[36-39]

Studies related to the role of oxidative stress in neurodegenerative diseases are limited with patients with AD and several studies demonstrated elevated levels of oxidative DNA damage and an impairment in the removal of oxidized purines in the lymphocytes of patients with AD.[16-19] A significant increase of 8OHG and an oxidized amino acid (nitrotyrosine) were detected in neurons of patients with AD suggesting that increased oxidative damage is an early event in AD that decreases with the progression of the disease.[40] The level of oxidative

damage and repair capacity in peripheral lymphocytes of AD patients and their age-matched controls was determined by the comet assay applied to freshly isolated blood samples with oxidative lesion-specific DNA repair endonucleases. Statistically significant elevation ($p<0.05$) of oxidized purines was observed in nuclear DNA of peripheral lymphocytes from 27 AD patients, compared to 12 age-matched control subjects, both at basal level and after oxidative stress induced by H_2O_2. AD patients also showed a diminished repair of H_2O_2-induced oxidized purines.[17]

PD is the second most prevalent age-related neurodegenerative disease after AD with physiological manifestations including tremors, bradykinesia, abnormal postural reflexes, rigidity and akinesia.[41] The pathogenic mechanism of the disease is not clear and oxidative stress seems to be one of the main etiological factors. In PD patients, the dopaminergic neurons are subjected to oxidative stress resulting from reduced levels of antioxidant defenses such as glutathione and high amount of intracellular iron. Several pro-oxidant conditions have been demonstrated in midbrain tissue from PD patients, including increased levels of iron and decreased levels of GSH, which can cause the increased appearance of oxidative products of lipids, proteins, and DNA.[4, 42, 43] The expression of high 8-oxoguanine DNA glycosylase (OGG1) levels has been found in PD patients as the expression of increased repair of DNA oxidative damage.[28] The increase of 8-oxo-guanine in mitochondrial DNA of PD patients as the occurrence of oxidative damage in nucleic acids has also been shown.[44]

In this study, the DNA damage as measured by comet assay was found to be significantly higher in the lymphocytes of the patients with PD compared to their healthy controls ($p<0.05$). Our findings are consistent with the very few studies done by comet assay. Petrozzi et al. performed the human lymphocyte micronucleus assay (HLMNA) and comet assay in 19 PD patients and 16 healthy controls.[26] PD patients showed a significantly increase in spontaneous MN frequency ($p<0.001$) and single strand break (SSB) levels ($p<0.001$) and oxidized purine base levels ($p<0.05$), supporting the hypothesis of oxidative stress as a relevant factor in the pathogenesis of PD. The presence of oxidative damage has been shown in leukocytes of untreated PD patients, supporting the hypothesis that PD is a systematic disorder. Migliorei and Coppede and Prigione et al. also showed higher level of oxidative stress in the peripheral lymphocytes from PD patients compared to healthy controls.[1,45] However in the lymphocytes of our patients with PD, no significant increase in DNA strand breakage was found after treatment with only H_2O_2 and with H_2O_2 and Fpg, compared to their healthy controls (Figure 1). In our study population, the majority of patients with PD (18 PD patients) had been treated with levodopa plus dopa-dicarboxylase (DDC) inhibitors such as benserazide and carbidopa for more than 5 years. Levodopa (LP) is widely used for the symptomatic treatment of PD, but its role in oxidative damage control is not clear. There have been controversial ideas about the toxicity of LD. Although it has been shown in some studies that LD treatment enhanced oxidative processes as measured by higher levels of lipoprotein oxidation in plasma and cerebrospinal fluid of PD patients[46], some recent studies even suggested a negative correlation between oxidative stress and LD dosage[45] showed that LD daily dosage is inversely correlated with ROS levels and positively associated with the activity of GSH reductase. Agil et al. also showed an inverse correlation between LD intake and plasma lipid peroxidation.[29]

Similar to our results, although the number of patients was limited to only nine patients compared to nine controls, Cornetta et al. observed that PD patients after 15h washout of the LD therapy showed high levels of DNA single strand breaks compared to healthy controls.[4] After the intake of LD therapy, a progressive and significant reduction of DNA damage up to 3 h from the administration of the appropriate dose is observed. It is also assumed that LD therapy exerts a long-lasting protection against oxidative stress in PD patients. The authors concluded that the accumulation of DNA lesions in PD patients was high, but these lesions decreased after LD intake. In addition, a recent paper by Oli et al. has demonstrated in vitro that

dopamine and L-DOPA possess an antioxidant capacity, but are also be able to increase the levels of 8-oxodG, an oxidative stres biomarker, without manifestation of chromosomal damage.[47] Zhu et al. hypothesized an adaptive response of neurons towards oxidative stress since they found that only few vulnerable neurons show clear signs of apoptosis, suggesting that the level of oxidative stress does not significantly exceed neuronal oxidative defenses.[48] They proposed that neurons of AD patients were exposed to low but chronic levels of oxidative stress that led them to elicit adaptive responses such as activation of stress-activated protein kinase pathways.

On the other hand, MS is a common, often disabling disease of central nervous system. Little is known about its cause or the factors that contribute to its unpredictable course. There is not enough study in the literature regarding the role of oxidative stress in patients with MS. Reactive oxygen species and nitric oxide intermediates are produced by activated macrophages and microglia and thus, they are likely candidates to be involved in tissue injury in multiple sclerosis.[49] Calabrese et al. support the role for nitrosative stress in the pathogenesis of MS and indicate that therapeutic strategies focused on decreasing production of NO by inducible NO synthase and/or scavenging peroxynitrite may be useful in alleviating the neurological impairments that occur during MS relapse. It is likely that peroxynitrite scavengers, specific inhibitors of iNOS, and/or modulators of GSH levels may be of use in therapy for MS, especially during relapse.[50] Oxidized lipids and oxidized DNA have been detected biochemically in brain tissue from patients with MS.[51-54] Haider et al. suggested profound oxidative injury of oligodendrocytes and neurons to be associated with active demyelination and axonal or neuronal injury in MS.[55] Satoh et al. reported that MS lymphocytes had a complex pattern of gene regulation that represented a counterbalance between promoting and preventing apoptosis and DNA damage of lymphocytes.[56] Our study is the first study that has examined the DNA damage in the lymphocytes of patients with MS by comet assay. In our study, although the basal DNA damage was higher in 22 MS patients compared to their 16 healthy controls, the difference was not statistically significant. However a significant increase in the tail moment which is a reliable indicator of DNA damage after treatment of lymphocytes with H_2O_2 and post-treatment with Fpg have been observed in the patients with MS compared to their healthy controls suggesting the susceptibility to oxidative DNA damage in these patients.

CONCLUSION

In conclusion, the data presented in this paper shows high level of DNA damage in the lymphocytes of patients with PD and MS, and indicates the possibility of oxidative stres in these neurodegenerative diseases. It is likely that antioxidants may be of use in therapy especially for MS. However, in order to make a definite conclusion and to investigate the possible mechanisms of these diseases, further studies are needed.

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