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Expression of 8-oxoguanine DNA glycosylase 1 (OGG1) and the level of p53 and TNF-alpha proteins in peripheral lymphocytes of patients with Alzheimer's disease

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Abstract

The aim of the study was to determine the extent of oxidative DNA damage (levels of 8-oxo2dG) and expression of OGG1 and p53 and TNF-α proteins in lymphocytes of Alzheimer's disease (AD) patients and a control group.
The studies were conducted on 41 patients with AD, including 25 women and 16 men aged 34-84 years. The control group included 51 individuals, 20 women and 31 men aged 22-83 years. The level of 8-oxo2dG was determined using HPLC/EC/UV, and the level of OGG1 and p53 and TNF-α proteins was determined with the Western blot method.
The results showed that both proteins participating in DNA repair (OGG1, p53) and the inflammatory protein TNF-α are involved in pathogenesis of neurodegenerative diseases. It also seems that a specific system for DNA repair (OGG1) may contribute to downregulation of the inflammatory factor (TNF-α) level, especially in the early stages of dementia. Moreover, the results showed that p53 protein can fulfil its function in DNA damage repair only in early stages of dementia.
It is possible that OGG1 and p53 and TNF-α proteins together or separately may be involved in pathogenesis of AD by repair of oxidative DNA damage and/or apoptosis.

Key words: *8-oxo2dG, OGG1, p53, TNF-α, peripheral lymphocytes, AD.*

Introduction

It has been accepted that oxidative stress and neuroinflammation can participate in the generation of reactive oxygen species (ROS) that can modify macromolecular structures such as DNA and in this way may play an important role in pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD) [3,4,35]. Among the markers of oxidative DNA modifications, 8-oxo-2'-deoxyguanosine (8-oxo2dG) is the most abundant product of hydroxyl-induced oxidation in the purine bases of nucleic acids. Variable levels of 8-oxo2dG have been reported in both brain [43,44] and peripheral blood lym-

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phocytes of AD patients [15,17]. Furthermore, it has been demonstrated that in humans, the repair of oxidized guanine within DNA involves 8-oxoguanine glycosylase 1 (OGG1). Up till now, a few isoforms of OGG1 have been identified and divided into two groups: type 1 (OGG1-1a, 1b, 1c) and type 2 (OGG1-2a, 2b, 2c, 2d, 2e) [30].

Studies by Dorszewska *et al*. [17] have shown that in peripheral blood lymphocytes of AD patients the expression of three isoforms of OGG1 (1a, 1b, 1c) changes similarly to the levels of 8-oxo2dG. Also, it was found that the OGG1-1c isoform appears to be significant in early stages of AD and all three type 1 isoforms (OGG1-1a, 1b, 1c) show a decrease in expression in AD patients with severe dementia. In the literature decreased expression of OGG1 enzyme in various areas of the brain has been reported, and in the study of Mao *et al*. [24] it may be affected by a mutation in the coding gene: C796 deletion or single amino acid substitution as well as a single point mutation GC-to-AT [19]. As shown [41], GC-to-AT transversion in the gene encoding OGG1 plays an important role not only in maintaining the stability of DNA, but also in immune system regulation and the development of many inflammatory diseases including autoimmune diseases and allergies. In addition, Mabley *et al*. [23] and Touati *et al*. [42] have demonstrated that in infected $OGG1^{-/-}$ mice the lack of OGG1 enzyme increases the protective effect against infection by significantly reducing the production of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and chemokines. It is well known that in the pathogenesis of AD several markers of inflammation are involved, e.g. increased levels of inflammatory cytokines (IL-1, IL-6, TNF-α, IL-8) and receptors for cytokines [27]. It is also believed that in AD patients inflammatory cytokines, such as TNF-α, are stimulated by activated microglia, leading to increased oxidative stress.

It is known that oxidized guanine in DNA induces GC-to-AT transversion-type point mutations. The same type of mutation was also observed in the tumour suppressor gene *p53* in human cancers [19]. According to de la Monte and Wands [12] p53 protein can be involved in neuronal death of AD patients, and its transcription is upregulated at the early stages of the disease and downregulated during the neurodegenerative process.

The purpose of this study was to determine the level of oxidative DNA damage in the form of 8-oxo2dG and analyse OGG1, p53 and TNF-α protein expression in peripheral blood lymphocytes of AD patients and healthy controls. In the treated groups the expression of isoform OGG1-1b, strongly associated with the level of OGG1 protein, was also analysed.

Material and methods

Patients

Forty-one AD patients, 16 males and 25 females, aged 34-84 years (72.5 ± 11.0 years, mean age ± SD), and 51 control subjects, 31 males and 20 females, 22-83 years of age (51.1 ± 21.0 years, mean age ± SD), were studied. Of these, 29 control subjects were younger than 60 years, 18 females and 11 males were 22-59 years old (35.8 ± 14.0 years, mean age ± SD), and 22 control subjects were older than 60 years, 13 females and 9 males aged 62-83 years (71.2 ± 5.5 years, mean age ± SD). The clinical diagnosis of AD was based on criteria of the National Institute of Neurological and Communicative Disorders-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [25]. The AD cases had mild (23-19 points), moderate (18-11 points) or severe (10-0 points) dementia, according to the results of the Mini-Mental State Examination (MMSE).

None of the control subjects had verifiable symptoms of dementia or any other neurological disorders.

The Local Ethics Committee approved the study and written consent of all patients or their caregiver was obtained.

Determination of 8-oxo2dG

Isolation of DNA

DNA was isolated from peripheral blood lymphocytes by fivefold centrifugation in a lytic buffer containing 155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4, in the presence of buffer containing 75 mM NaCl, 9 mM Na_2EDTA, pH 8.0, and sodium dodecyl sulfate and proteinase K (Sigma, St. Louis, MO). Subsequently, NaCl was added, the lysate was centrifuged, and DNA present in the upper layer was precipitated with 98% ethanol.

Enzymatic hydrolysis of DNA to nucleosides

DNA was hydrolyzed to nucleosides using P_1 nuclease (Sigma), for 2 h at 37°C in 10 mM NaOAc, pH 4.5. The solution was buffered with 100 mM Tris-HCl,

pH 7.5. Subsequently, the DNA was hydrolyzed with alkaline phosphatase (1 U/µl; Roche, Germany) for 1 h at 37°C and the obtained nucleoside mixture was applied to a high performance liquid chromatography system with both electrochemical and ultraviolet detection (HPLC/EC/UV).

Estimation of 8-oxo2dG

To determine 8-oxo2dG levels, the nucleoside mixture was applied to the HPLC/UV system (P580A; Dionex, Germany) coupled to an electrochemical detector (CoulArray 5600; ESA, USA). Nucleosides were separated in a Termo Hypersil BDS C18 (250 x 4.6 x 5 µm) column (Germany). The system was controlled, and data were collected and processed using Chromeleon software (Dionex, Germany). The results were expressed as the ratio of oxidized nucleosides in the form of 8-oxo2dG to unmodified 2'dG [33].

Estimation of OGG1 and TNF-α and p53 protein levels

Isolation of protein

Blood was layered onto Gradisol L at a 1 : 1 ratio and centrifuged, followed by collection of the interphase which was then rinsed in PBS buffer (0.9% NaCl in phosphate buffer) and centrifuged. The obtained lymphocyte precipitate was rinsed with radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1% IGEPAL CA-630, 0.05% SDS, and 1% sodium deoxycholate), supplemented with a protease inhibitor cocktail (Sigma) and homogenized in a mixture of RIPA with protease inhibitor cocktail (16 : 1) and 0.5 µl PSMF (Sigma) in isopropanol (10 mg/100 µl), centrifuged, and the obtained supernatant underwent further analysis [32].

Western blot

The OGG1 and THF-α proteins were analysed in 12% and p53 protein in 7.5% polyacrylamide gel. Equivalent amounts of protein (30 µg protein/lane) were loaded into the wells. The gel-separated proteins were electrotransferred to nitrocellulose filter in semidry Western blot analysis apparatus (Apelex, France). To estimate the levels of the OGG1 protein, the filters were exposed first to an anti-OGG1/2 goat polyclonal antibody (G-20, IgG, 200 µg/1.0 ml; Santa Cruz, USA), and the THF-α protein, anti-THF-α goat polyclonal antibody (N-19, IgG, 200 µg/1.0 ml; Santa Cruz, USA), and the p53 protein, anti-p53 mouse monoclonal antibody (IgG-2a, 200 µg/1.0 ml; Santa Cruz, USA) diluted 1 : 500.

Subsequently, individual sheets of nitrocellulose filter were incubated with the second antibody, for OGG1 and THF-α proteins, mouse anti-goat IgG-HRP (200 µg/0.5 ml; Santa Cruz, USA), and for p53 protein goat anti-mouse IgG-HRP (200 µg/0.5 ml; Santa Cruz, USA) at a dilution of 1 : 2000. To stain immunoreactive bands, peroxidase BMB was added (BM blue POD substrate precipitation; Roche, Germany). The surface area of the immunoreactive bands was registered using a densitometer (GS-710; Bio-Rad, Hercules, CA) in the Quantity One System.

Statistical analysis of results

The data were evaluated using the Mann-Whitney nonparametric test for independent variables.

Levels of biochemical parameters at particular stages of AD development were evaluated using the parametric one-way ANOVA for independent variables.

Normal distribution was evaluated using the Kolmogorov-Smirnov test.

Correlations between the obtained results were tested using the Spearman test.

The programs GraphPad (Instant, USA) and Statistica for Windows (StatSoft, USA) were used to perform statistical analysis of the results.

Results

Levels of oxidative DNA damage in the form of 8-oxo2dG and expression of proteins responsible for DNA repair (OGG1, p53) and the inflammatory process (TNF-α) were examined in peripheral blood lymphocytes of AD patients and the control group.

This study demonstrates that, after the 60th year of life, no significant alterations were detected in the DNA oxidative modification product 8-oxo2dG in peripheral blood lymphocytes (Table I). In addition, at this age oxidative DNA changes were accompanied by a statistically significant decrease in levels of protein involved in DNA repair, OGG1 (Mann-Whitney test, $p < 0.001$ compared to the controls before 60 years of age) and the inflammatory process, TNF-α (Mann-Whitney test, $p < 0.05$ compared to the controls before 60 years of age), along with a simultaneous increase in the level of DNA repair protein, p53 (Mann-Whitney test, $p < 0.05$ compared to the controls be-

fore 60 years of age). Moreover, it was found that only controls who did not exceed the limit of physiological aging (controls younger than 60 years) manifested a positive correlation (R Spearman, +0.4213) between the DNA repair protein (p53) and the proinflammatory protein (TNF-α) (Spearman rank test, $p < 0.05$).

However, in the peripheral blood lymphocytes of AD patients (Table II) there was a significant increase (Mann-Whitney test, $p < 0.05$ compared to the controls) in the level of the DNA oxidative damage product 8-oxo2dG. Significantly decreased levels of OGG1 and TNF-α proteins were also observed in the AD patients (Mann-Whitney test, $p < 0.05$ compared to the controls), with a parallel insignificant increase in p53 protein levels. Additionally, it was found that in AD patients an inverse correlation exists (R Spearman, –0.3632) between the two DNA repair proteins OGG1 and p53 (Spearman rank test, $p < 0.05$).

It was also found that the levels of 8-oxo2dG fluctuated as the disease progressed (Table III), leading to an insignificant increase in 8-oxo2dG from mild to moderate dementia, while in severe dementia the levels of 8-oxo2dG tended to remain stable although higher than in healthy controls (Fig. 1). In the stage of mild to moderate dementia along with the increase of oxidative DNA damage, there was an increase in OGG1 protein level (one-way ANOVA test, $p < 0.05$ between patients with mild and moderate dementia), reaching control values in the moderate stage and decreased in conditions of deep dementia. In contrast, the p53 protein levels in each stage of AD development remained statistically nonsignificantly higher compared to the controls, with a tendency for a decrease in the progress of dementia. In addition, TNF-α protein levels at any stage of AD development remained insignificantly lower com-

Table I. Levels of DNA oxidative damage (8-oxo2dG/dG × 10^{-5}) and expression of OGG1 and p53 and TNF-α proteins in peripheral blood lymphocytes in the two control groups

Compound	Control individuals 22-59 years of age	Control individuals 62-83 years of age	*P*
8-oxo2dG	24.4 ± 9.8	30.1 ± 18.2	0.2201
OGG1 protein	60.0 ± 18.5	42.1 ± 15.0***	0.0007
p53 protein	64.6 ± 16.4	73.9 ± 22.6*	0.0500
TNF-α protein	46.5 ± 12.6	37.7 ± 19.1*	0.0200

The results represent the ratio of 8-oxo2dG/dG x 10^{-5}. Protein levels are given in conventional units, % radioactive surface bands.
Mean ± SD; Mann-Whitney test.
*Statistical significance at *p < 0.05, ***p < 0.001*
Control younger than 60 years.
Statistical significance at p < 0.05 (p = 0.0321)
Spearman coefficient +0.4213 between the expression of p53 protein and TNF-α protein

Table II. Levels of DNA oxidative damage (8-oxo2dG/dG x 10^{-5}) and expression of OGG1 and p53 and TNF-α proteins in peripheral blood lymphocytes in AD patients and the control group

Compound	Control individuals 22-83 years of age	AD patients 34-84 years of age	*P*
8-oxo2dG	27.4 ± 14.9	34.3 ± 19.5*	0.0500
OGG1 protein	51.4 ± 18.8	41.2 ± 15.1**	0.0078
p53 protein	68.8 ± 19.8	73.1 ± 19.8	0.2106
TNF-α protein	42.5 ± 16.3	36.6 ± 12.2*	0.0338

The results represent the ratio of 8-oxo2dG/dG x 10^{-5}. Protein levels are given in conventional units, % radioactive surface bands.
Mean ± SD; Mann-Whitney test.
*Statistical significance at *p < 0.05, **p < 0.01*
Patients with AD.
Statistical significance at p < 0.05 (p = 0.0485)
Spearman coefficient –0.3632 between the expression of OGG1 protein and p53 protein

Table III. Levels of DNA oxidative damage (8-oxo2dG/dG × 10^{-5}) and expression of OGG1 and p53 and TNF-α proteins in peripheral blood lymphocytes compared to the extent of dementia (MMSE) in AD patients

Compound	Mild dementia 23-19 points on MMSE	Moderate dementia 18-11 points on MMSE	Severe dementia 10-0 points on MMSE	*p*
8-oxo2dG	30.5 ± 12.4	34.2 ± 23.6	34.5 ± 19.4	0.8596
OGG1 protein	32.2 ± 5.5	51.0 ± 16.4*	39.7 ± 14.9	0.0500
p53 protein	81.9 ± 6.2	72.3 ± 21.2	70.1 ± 22.2	0.4696
TNF-α protein	39.2 ± 11.3	33.0 ± 13.0	37.3 ± 12.5	0.6100

The results represent the ratio of 8-oxo2dG/dG x 10^{-5}. Protein levels are given in conventional units, % radioactive surface bands.
Mean ± SD; one-way ANOVA test.
*Statistical significance at *p < 0.05, between patients with mild and moderate dementia.*

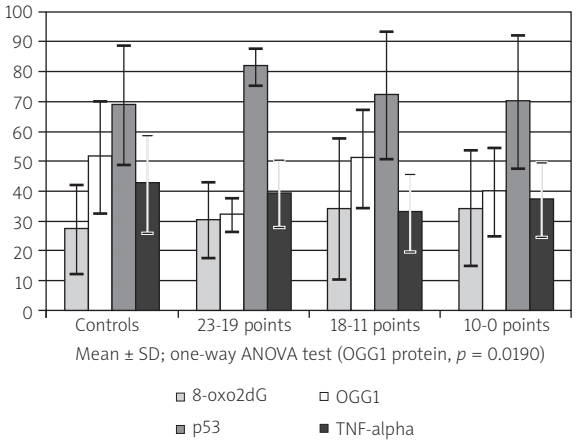

Fig. 1. Levels of DNA oxidative damage (8-oxo2dG/dG × 10^{-5}) and expression of OGG1 and p53 and TNF-α proteins in peripheral blood lymphocytes compared to the extent of dementia (MMSE) in AD patients and in control group.

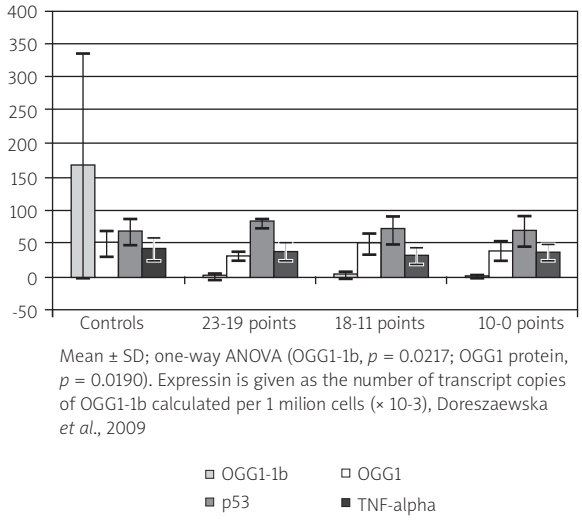

Fig. 2. Levels of DNA oxidative damage (8-oxo2dG) and expression of OGG1 protein and p53 protein and TNF-α protein in peripheral blood lymphocytes compared to the extent of dementia (MMSE) in AD patients and in control group.

pared with controls, although it reached the lowest expression in the patients with the highest levels of OGG1 protein.

In the individuals studied, the expression of the OGG1-1b isoform, most strongly associated with the level of OGG1 protein, was also analysed (Fig. 2). Its highest level of expression was observed in the group of patients with the lowest levels of proinflammatory factor, TNF-α.

Discussion

In degenerative diseases, including AD, neuronal degradation occurs via weakening of the function of several key mitochondrial enzymes, damage of mitochondrial structures and also by increasing oxidative stress, which decreases the effectiveness of endogenous antioxidant mechanisms [18,21,34]. It has been shown that an environment rich in ROS can modify macromolecular structures such as DNA and RNA [5,26]. One of the markers of oxidative DNA modification is oxidized DNA guanine or its nucleoside, 8-oxo2dG. 8-oxoguanine is considered to be a marker of oxidative DNA damage in degenerative diseases as well as aging processes.

Studies by Dorszewska *et al*. [17] have shown that peripheral blood lymphocyte levels increase in about 40% of the population after 60 years of age. An increased level of 8-oxo2dG has also been

demonstrated in the aging brain between the ages of 43 and 91 [1].

It is known that oxidative damage of DNA may cause mutations in the nucleic acids, leading to a reduction in the viability of many body organs and symptoms of disease, including neurodegenerative diseases such as AD [2].

As shown in studies, oxidative DNA damage in AD may occur both in the central nervous system [43,44] and in peripheral blood lymphocytes [15-17,28].

Nunomura *et al.* [31] and this study show that the level of a marker of oxidative stress depends on the stage of dementia. We have also demonstrated that the oxidative DNA damage (8-oxo2dG) level in peripheral blood lymphocytes of patients with AD increases as dementia progresses from mild to moderate, and shows a tendency to remain unchanged at the level of dementia according to the MMSE score. Nunomura *et al.* [31] suggest that oxidative DNA damage increases only during the early stages of the disease and then decreases with the progression of the disease due to activation of a compensatory system.

Moreover, it is believed that the increase in oxidatively altered bases in the DNA is the weakening of a specific repair system of oxidized guanine both in AD and physiological aging processes [20].

This study and Dorszewska *et al.* [17] demonstrated that it may be influenced by the decrease in the level of OGG1 protein and by the decrease in mitochondrial OGG1-1b isoform expression. Further studies by Iida *et al.* [20] indicate that the reduction in OGG1 expression in the brain of patients with AD is accompanied by the formation of neurofibrillary tangles, axonal dystrophy and reactive astrocytes. However, Mao *et al.* [24] have demonstrated that the decreased excision activity of OGG1 in patients with AD may be affected by mutations in the gene encoding OGG1. In that study, the presence of mutations was found in 4 of 14 patients with AD; two of these were C796 deletions that completely eliminate the activity of the OGG1 enzyme, and two were single point mutations that lead to distinctly decreased activity of OGG1 glycosylase. It seems, however, that partial or complete inactivation of OGG1 glycosylase in some disease states may also have a protective role. As has been demonstrated in studies by Mabley *et al.* [23] and Touati *et al.* [42] carried out on infected OGG1$^{-/-}$ deficient mice, inactivation of OGG1 enzyme increases the protective effect and reduces the inflammatory response due to a marked decrease in production of proinflammatory cytokines such as TNF-α.

According to the authors, in the presence of the infection generation of oxidative DNA damage in the inactivation of OGG1 glycosylase necessary for DNA repair occurs in less severe gastric inflammation of mice, probably due to stronger regulation of gene expression of inflammatory factors. It appears that there is a correlation between the efficiency of the DNA repair system and development of inflammation associated with the production of Th1 cytokines and the pathogenesis of inflammatory, autoimmune and allergic diseases and possibly AD. It is known that in the pathogenesis of AD involving proinflammatory Th1 cytokines such as IL-1, IL-6, TNF-α, IL-8, and receptors for cytokines produced and secreted by activated microglia surround the developing amyloid plaques in the brain of patients [9]. It is also believed that markers of inflammation in AD patients can be a cause as well as a consequence of ROS and nitrate generation [11,27], which may affect the transcription of proinflammatory genes in glial cells and lead to activation of β-secretase, stimulating more amyloid production and thus triggering a cascade of neurotoxic events [38]. It is believed that the overexpression of brain cytokines may affect their peripheral distribution, and that peripheral cytokines can interact with cells of the central nervous system. In addition, it has been demonstrated that one of the cytokines, TNF-α, plays an important role in the development of AD neuroinflammation [46,47]. TNF-α appears to be nonspecific, but a strong factor in the development of cardiovascular disease, hypertension, some psychiatric illnesses, including depression and dementia, and impairment of cognitive function [40].

It has also been shown that TNF-α may induce both pro-apoptotic and anti-apoptotic responses in neuronal cells. In vitro studies have shown that TNF-α, as a proinflammatory factor, promotes cell death by apoptosis in the neuronal cell line. However, there are also studies indicating that TNF-α action is transduced through binding to TNF receptor I and can promote both cell death and the survival of cells [9,22]. Data on the level of TNF-α in AD are mixed. Most of the authors have reported that the level of TNF-α in cerebrospinal fluid and blood serum was unchanged [36,37]. In another study it was found that increased levels of this cytokine

appear in both the brain and the plasma of AD patients [39]. An increase in plasma TNF-α was observed in the studies by De Luigi *et al.* [13] and Zuliani *et al*. [47]. But other studies have found a decrease in the level of TNF-α in peripheral blood lymphocytes as well as plasma in AD patients (this study, [45]). Other studies and the literature indicate that the level of TNF-α depends on genetic factors and that G308A polymorphisms of TNF-α predispose to overexpression of this cytokine [10,46]. However, this study shows that the level of TNF-α probably also depends on OGG1 expression, especially in the early stage of dementia.

It also seems that the decreased TNF-α levels after 60 years observed in this study, together with the reduced OGG1 level, may be important for the immune response in the aging process and also may be associated with decreased risk of diseases of old age [6].

It has been demonstrated that inflammatory factors may lead to the generation of oxidative stress and oxidative DNA damage [42], which can induce GC-to-TA, TA-to-AT, and GC-to-AT type transversion in DNA repair enzymes, both in glycosylase OGG1 and in the *p53* gene [19].

There have been reports in the literature indicating that p53 protein participates in neuronal apoptosis in the brain of AD patients [12]. Probably it is associated with increased expression of p53. An increase in the level of p53 protein has been observed both in cultures of human and rat neurons and astrocytes, as well as in peripheral blood lymphocytes and brain of patients with AD (this study, [15]).

It is believed that both 1-42 amino acids β-peptide form and presenilin 2 decreasing the level of presenilin 1 are responsible for the increase in p53 expression in AD [14,29]. As has been demonstrated in the study by De la Monte and Wands [12] and also in this study, the highest expression of p53 protein occurs in early stages of AD, and tends to decrease in developing dementia, probably due to oxidative modification leading to changes in the activity of p53 protein, which acts as a DNA repair protein [7,8].

It is also suggested that an increased level of p53 protein in patients without dementia features between the age of 62 and 83 years is involved in the repair of oxidative DNA damage rather than in neuronal degradation.

Both DNA repair proteins (OGG1, p53) and the inflammatory protein TNF-α are involved in the pathogenesis of degenerative disorders, as shown in this study. It also seems that a specific system for DNA repair (OGG1) may contribute to downregulation of TNF-α expression, especially in the early stages of dementia. It also appears that only in the early stage of dementia can p53 protein fulfil its function in DNA repair.

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