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Determination of possible genotoxic damage by comet assay in patients under treatment due to depression and anxiety disorders

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Abstract

Anxiety and depression, very common disorders today, are often seen together. The present study was designed to detect DNA damage in patients diagnosed and treated with depression and anxiety using the Comet test. Thirty-eight patients who received pharmacological therapy (selective serotonin reuptake inhibitors-SSRIs, selective norepinephrine reuptake inhibitors-SNRIs, and antipsychotics) for anxiety and depression and twenty-eight healthy volunteers were included in the study. The comet test was used to evaluate the DNA damage in peripheral blood lymphocytes. As a result, DNA damage in patients in all the treatment groups was higher than those in the control group. It was determined that factors such as age, gender, initial or recurrent diagnosis, SNRI or SSRI use, and fluoxetine use did not cause a statistically significant difference in DNA damage in patient groups. This study revealed that both depression and anxiety disease and the treatment of the disease may present a risk factor for DNA damage.

Keywords: *Depression, anxiety, comet assay, genotoxicity.*

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Depresyon ve anksiyete tedavisi gören hastalarda comet testi kullanılarak olası genotoksik hasarın belirlenmesi

Öz

Anksiyete ve depresyon, günümüzde çok sık görülen rahatsızlıklar olup sıklıkla birlikte görülmektedir. Bu çalışma, comet testi kullanılarak depresyon ve anksiyete bozukluğu tanısı alan ve tedavi edilen hastalarda DNA hasarını ölçmek için tasarlanmıştır. Anksiyete ve depresyon nedeniyle farmakolojik tedavi (seçici serotonin geri alım inhibitörleri-SSRI'lar, seçici norepinefrin geri alım inhibitörleri-SNRI'lar ve antipsikotikler) alan otuz sekiz hasta ve yirmi sekiz sağlıklı gönüllü çalışmaya dahil edilmiştir. DNA hasarını değerlendirmek için periferal kan lenfositlerinde comet testi kullanılmıştır. Sonuç olarak tedavi gruplarındaki tüm hastalarda DNA hasarı kontrol grubuna kıyasla daha yüksek bulunmuştur. Hasta grubunda yaş, cinsiyet, ilk veya tekrarlayan tanı, SNRI veya SSRI kullanımı ve fluoksetin kullanımı gibi faktörlerin DNA hasarında anlamlı bir farklılığa neden olmadığı bulunmuştur. Bu çalışma, hem depresyon ve anksiyete hastalığının hemde hastalığın tedavisinin DNA hasarı açısından bir risk faktörü oluşturabileceğini ortaya çıkarmıştır.

Anahtar kelimeler: *Depresyon, anksiyete, comet testi, genotoksisite.*

1. Introduction

Depression is a common and treatable medical illness that negatively affects how a person feels, thinks, and behaves [1-2]. Depressive disorders are frequent and cause increased healthcare, treatment costs, and labor loss [1, 3-5]. Depression approximately affects 1 in 15 adults in any given year. In addition, 1 in 6 people experiences depression at some time in their life. Depression can occur at any age; however, it first appears in the mid-20s on average. It is more common in women than in men, and it ranks 4th in terms of disease destruction. It causes more disability than ischemic heart disease and cerebrovascular disease [1-2]. The presence of comorbidities in patients with depression is 73.3%, and all anxiety disorders are 56% [6]. Anxiety and depression disorders are common disorders and often occur together [7-9].

Pharmacological treatment, structured psychotherapy, and relaxation training are among the methods used in the treatment of depression and anxiety disorders. While interpersonal psychotherapy, cognitive-behavioral psychotherapy, or problem-solving therapies are used in mild depression, antidepressant medication is required in moderate and severe depression. SSRIs (selective serotonin reuptake inhibitors) and SNRIs (serotonin noradrenaline reuptake inhibitors) are a class of medications effectively used in treating depression and anxiety [5, 10-12].

In this study, the comet assay, known as alkaline single cell gel electrophoresis (SCGE), was used to determine DNA damage in patients with anxiety and depression who were under pharmacological treatment. This assay is a simple, reliable, easy, fast, cheap, and sensitive technique requiring a small number of cells [13-17]. The comet assay can detect DNA single- and double-strand breaks and alkali-labile sites in alkaline conditions without the requirement for cell division. It is increasingly used in human

biomonitoring and occupational studies [18-20]. Increased tail length as the indicator of DNA damage [15, 21]; if unrepaired it is mutagenic, contributes to genomic instability, and increases the risk of some types of cancer incidence [22].

It is important to investigate the genotoxic risk of active ingredients of drugs in unbiased laboratories [23-24]. Many drugs might be carcinogenic due to mechanisms associated with their genotoxicity; therefore, it is crucial to examine the advantages or disadvantages of long-term drug treatments [24]. For this purpose, researchers are trying to determine the possible clastogenic, mutagenic, and genotoxic effects of diverse active ingredients of drugs by various short-term *in vitro* and *in vivo* tests [23, 25-27]. Studies have shown that many active ingredients of drugs may have genotoxic effects [23, 28-34]. Studies on SSRIs and SNRIs have concluded that their active ingredients have no genotoxic effects [35-37], but some drug active ingredients have been suggested as toxic in many investigations [25, 27, 38-40]. Studies on the effects of anxiety and depression treatment on DNA damage are very limited and have shown controversial results. Some of these studies explained that antidepressants such as escitalopram, doxepin, sertraline, and duloxetine or atypical antipsychotic drugs such as olanzapine, risperidone, and calctiapine did not increase DNA damage; however, they could be genotoxic and cytotoxic in high doses [26, 36, 37, 39, 41]. Other studies reported that DNA damage would be higher in anxiety and depression patients [42], and drugs like reboxetine, venlafaxine, milnacipran, trazodone, and nefazodone cause DNA damage in patients [25, 27, 34, 38, 40]. In another study conducted by Andreazza et al. (2007), DNA damage was found to increase using the comet test in patients with bipolar mood disorders compared to the control group. Besides, this damage was related to the severity of disease symptoms [18]. Similarly, methylphenidate (MPH) used in the treatment of attention deficit and hyperactivity in children was shown to increase the formation of liver tumors in mice [43]. In another study conducted with the comet test in mice exposed to the same active ingredient, MPH did not acutely increase DNA damage, but chronic treatment increased DNA damage [44].

Many studies in the literature address the relationship between anxiety and depression drugs and cancer incidence [45-49]. On the other hand, some of these investigations indicated that the use of antidepressants does not increase the risk of breast cancer [46, 48-49]. Due to these contradictory results, in our study, possible genotoxic damage in patients who had a diagnosis of anxiety and depression disorder and had medical treatments with some drugs was investigated using the comet assay. It is important to describe these drugs in terms of the genotoxicity as safe and reliable for patient. Therefore, this study aims to determine whether the anxiety and depression disease and the drugs used in the treatment cause any DNA damage in patients' lymphocytes.

2. Material and methods

2.1. Patients

Isolated lymphocytes obtained from the whole blood of thirty-eight patients aged 20-50 who were treated for anxiety and depression for at least three months were used. Twenty-eight volunteers were also included in the study as the control group who did not have any chronic health problems and at the same age group as the patients. The patients were diagnosed according to DSM-IV criteria. Among the patients, 29 received SSRIs (single drug users, n=13, combined drug users, n=16), 8 received SNRIs (single

drug users, n=6, combined drug users, n=2) and 1 patient received antipsychotic and noradrenergic dopaminergic reuptake inhibitor together. The details of the medications of the patients are shown in Table 1.

The patient group that has been diagnosed with anxiety and depression was selected randomly and agreed to participate in the study. They were categorized according to their age, gender, use of single or combined drugs, use of fluoxetine, SSRIs or SNRIs, first-time diagnosis, or recurrent diagnosis. Demographic data was collected from all volunteers with a questionnaire on detailed information about their health status. They had no any other chronic disease.

The patients and healthy volunteers as a control group signed a consent form to participate in the study.

2.2. Comet assay

Lymphocytes were isolated using the method applied by Hininger et al. (2004) after modifications and stored at -20°C. Then 100 µL of blood samples were diluted in 1000 µL PBS and kept on ice for 10 minutes. Lymphocytes were isolated using Biocoll separating solution at the eppendorf tubes. Later, the tubes were centrifuged at +4°C at 1060 rpm for 3 minutes. The cloudy layer formed in the middle of the tube was removed for lymphocyte isolation. The mix of RPMI / FCS (9: 1) and DMSO (9:1) was added to the isolated lymphocytes and was suspended. The isolated lymphocytes were stored at -20°C for 45-60 days [50]. Comet assay was performed by the method used by Singh et al. in 1988, with some modifications. All the steps in the comet assay were conducted in dim light to avoid further nonspecific DNA damage. The samples stored at -20°C were taken to room temperature and dissolved on the test day. Lymphocyte suspensions were centrifuged at 3000 rpm for 5 minutes and the supernatant was removed. Trypan blue exclusion test was used to determine cell vitality. The comet assay was performed after the viability rate was determined to be 94%.

Lymphocytes were re-suspended in PBS. Low-melting-point agarose (0.65%) was mixed with lymphocytes and dropped on slides previously coated with normal-melting-point agarose (0.65%). In this way, the lymphocytes were embedded between two agars like a sandwich model. The slides were covered with coverslips and kept at +4°C for 20-25 minutes. Then the coverslips were removed and kept for 1 hour at +4°C in a refrigerator in lysing solution adjusted to pH 10 (2.5 M NaCl, 100 mM EDTA, 10 mM Tris). The slides were then kept in a tank filled with electrophoresis buffer (pH = 13) for 20 minutes for DNA unwinding. After that the DNA was electrophoresed for 20 minutes at 25 V, 300 mA. At last, the slides were kept in a neutralization buffer (0.4 M Tris, pH = 7.5) for 5 minutes twice. The slides were stained by ethidium bromide and covered with a coverslip [51-52].

2.3. Lymphocyte count and comet evaluation

A total of 200 lymphocytes from each patient and volunteers were evaluated using the "Comet Assay IV, Perceptive Instruments Ltd., UK" at 400x magnification under a fluorescent microscope (546 nm excitation and 590 nm barrier filter). The statistical significance of the results was examined in terms of tail length, % tail intensity, and tail moment.

2.4. Statistical analysis

The statistical analysis was performed using the Mann-Whitney U test since the data obtained in this study was not normally distributed. A p-value of less than 0.05 was considered significant.

3. Results

When the patient and control groups were compared, DNA damage was found to be higher in the patient group under treatment in terms of all the parameters (tail length, tail intensity, and tail moment) (Figure 1, Table 2). These differences were also statistically significant ($p<0.01$). However, when the comet results were evaluated within the patient group by age (20-35 years old, 36-50 years old) and gender, there was no statistically significant difference. Similarly, there was no statistically significant difference in terms of DNA damage between patients with the first-time diagnosis and recurrence. Furthermore, there was no statistically significant difference in the comet assay results between patients receiving SSRIs and SNRIs (Table 2).

When patients under monotherapy or combined treatment were compared, DNA damage was higher in patients using a single medication. However, this difference was statistically significant only in the tail moment value ($p = 0.04$) (Table 2). In addition, there was no difference in the comet parameters between patients receiving fluoxetine monotherapy and single-drug therapy other than fluoxetine. Also, no difference was detected between patients taking a combinations therapy with and without fluoxetine, in terms of DNA damage. Furthermore, there was no difference in the comet parameters between patients receiving fluoxetine monotherapy and other single-drug therapy. When the comet results of the patients who were under fluoxetine (mono + combined) therapy and those who were not were compared, no difference was observed in DNA damage (Table 2).

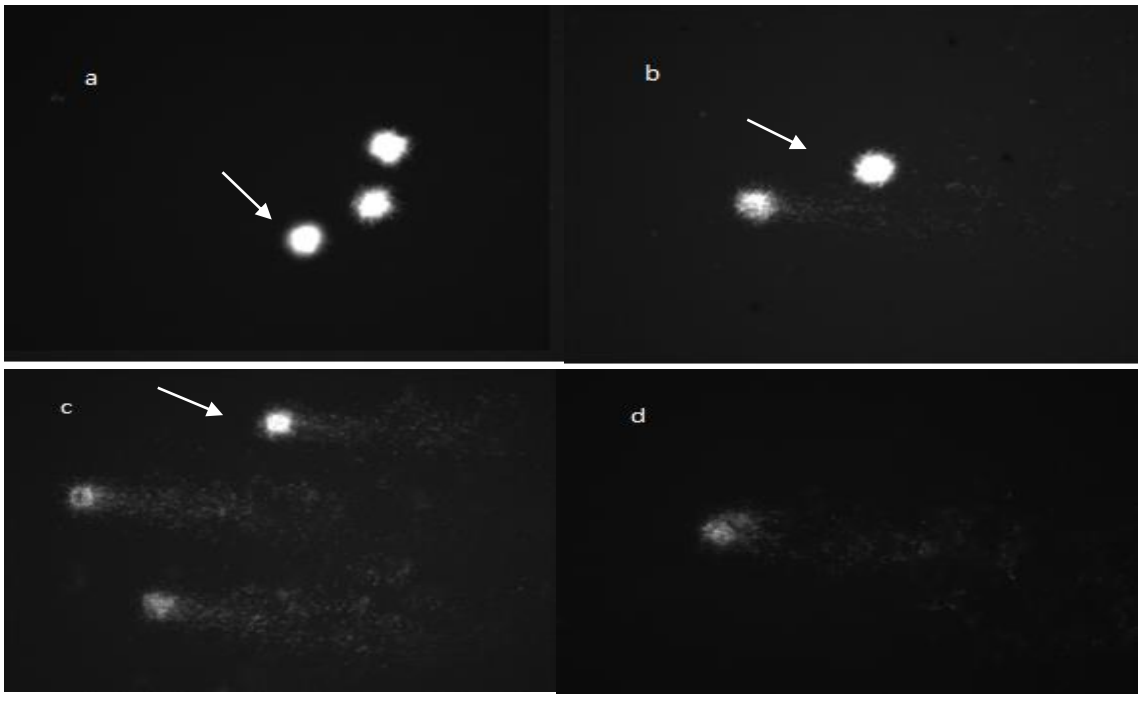

Figure 1. Representative comet images of isolated human lymphocytes. a: Undamaged DNA, b: Slightly damaged DNA, c: Moderately damaged DNA, d: Very damaged DNA

4. Discussion

Many drugs can be carcinogenic due to mechanisms associated with genotoxicity. Therefore, it is important to examine the advantages and disadvantages of long-term drug treatments [24]. For this reason, scientists are working on the possible clastogenic, mutagenic, and genotoxic effects of various drug active ingredients by *in vitro* and *in vivo* test methods [23, 25-27].

In our study, comet assay was used to determine possible DNA damage in the patient groups under pharmacological therapy for at least three months due to anxiety disorder and depression. Assessment of DNA damage using the comet assay is a biomarker of genotoxicity [53]. This assay is a reliable test revealing particular biomarkers such as single and double-strand breaks, alkaline labile regions, DNA-DNA/DNA-protein cross-links, and incomplete excision repair sites [15, 54-57]. In the Comet assay, undamaged DNA appears as a "head" while fragmented DNA that involves single and double-strand breaks move faster and forms the "Comet tail" [15, 58-60]. Tail length, intensity, and moment parameters are frequently used to determine DNA damage following exposure to various genotoxins [26, 51, 52, 61].

Our findings revealed that DNA damage determined by the comet test was higher in the patient group when compared to the control in terms of tail length, intensity, and moment. In a study carried out in schizophrenic patients, the sensitivity of lymphocytes exposed to external factors such as hydrogen peroxide and gamma-lights was evaluated using the comet test. There was no difference in terms of DNA damage between these patients and the normal population [62]. Another study conducted on 25 patients diagnosed with depression and under citalopram treatment, 20 patients under sertraline treatment, and 14 healthy volunteers revealed that DNA damage evaluated by the comet assay significantly reduced ($p < 0.001$) after 15 weeks of treatment by citalopram and sertraline (in our study 8 patients used sertraline between 50-100 mg/day dose range) [26]. In addition, there was no difference in the comet parameters between patients receiving sertraline and drug therapy other than sertraline in our study. In contrast, in a study performed in patients diagnosed with anxiety and depression, DNA damage was significantly higher than the control group which was similar to our findings based on the comet assay [42]. On the contrary, some studies have reported no DNA damage following antipsychotics and SSRIs treatments [37, 39, 41]. In a study performed *in vitro* human lymphocytes, atypical antipsychotic drugs olanzapine, risperidone, and calctiapine, at between 0-250 mg/L, did not cause DNA damage. However, they induced cytotoxic effects in higher doses (>250 mg/L) [41]. Similarly, in another *in vitro* experiment, exposure to escitalopram, an SSRI drug used in the treatment of many psychiatric diseases, and doxepin, a tricyclic antidepressant (1, 2.5, 5, and 10 μg/mL), did not cause any significant increase in DNA damage and MN formation in human lymphocytes. The authors concluded that high doses of doxepin and escitalopram may cause a potential genotoxic risk [39]. In our study, six patients used escitalopram in the 10-15 mg/day dose range. When the patient groups under SSRI treatment were compared with the control group, there was a statistically significant increase in the comet parameters examined in our study.

In a study, the effect of a single antidepressant treatment (amitriptyline, imipramine, or fluoxetine) was compared with combined treatment including amitriptyline/imipramine+fluoxetine in mouse neuronal cell culture. The effects of

these drugs were evaluated for 48 and 96 hours, and it has been found that the use of a single antidepressant for 48 hours increased the apoptotic cell death dose-dependently by activation of the caspase-3 pathway. The authors concluded that monotherapy with antidepressants was more toxic than combined treatment [27]. Our results also revealed that DNA damage was higher in patients using a single drug over the patients using a combined treatment which is coincident with the previous investigation. In contrast, Pereira et al. (2009) have stated that subacute exposure to the antidepressant duloxetine did not affect the values of the comet test in mice (10 or 20 mg/kg for 5 days); therefore, it was not reported as genotoxic [36]. In contrast to our results, in a study on the acute (24 hours) and chronic (14 days) effects of sertraline, among widely prescribed drugs, at low, medium, and high doses, 10, 40, and 80 mg/kg, respectively, there was no statistically significant difference in comet test data (tail length, intensity, and moment) in the rats exposed to sertraline, but acute administration caused slightly more DNA damage compared to chronic treatment [37]. In another different study, 10 patients with generalized anxiety disorder and major depression under sertraline treatment (50 mg daily from 10 months to 1 year), 18 non-treated patients with similar psychopathology, and 14 healthy volunteers were used. Here, the frequency of chromosome aberrations and sister chromatid exchanges (SCEs) were not significantly different in sertraline-treated and non-treated patient groups. However, sertraline-treated and non-treated patient groups showed higher frequencies of SCEs than the healthy controls. This difference may be explained by psychogenic stress [35].

There are different studies on the DNA damage that occurs with depression and anxiety disorder and during the treatment of these diseases. Czarny et al. (2018) reported that elevated levels of 8-oxoguanine, a marker of oxidative DNA damage, were found in the lymphocytes, urine, and the serum of the patients suffering from clinical depression and/or depression comorbid with other non-psychiatric diseases and that elevated levels of reactive oxygen and nitrogen species may lead to increase in DNA damage. Furthermore, it was shown that the patients' cells repaired peroxide-induced DNA damage less efficiently than controls. Therefore, oxidative stress and less efficient DNA damage repair may lead to increased DNA damage in depressed patients [63]. Antidepressants contain two potentially hazardous components in their chemical structures; one of these is the aromatic ring, and the other is the nitro group [34, 64, 65]. The nitro group may be converted into nitroso compounds which in turn may form alkylating molecules [31, 34, 66]. Alkylating agents are electrophilic compounds that show an affinity for nucleophilic centers of DNA or proteins resulting in the covalent transfer of an alkyl group [34, 67]. The alkylation of DNA by nitroso compounds induces a variety of lesions (e.g. DNA adducts, cross-links, and strand breaks) [68]. In contrast, it was also reported that SSRIs, which are drugs used in the treatment of depression, act as anti-inflammatory agents and antioxidants that can reduce DNA strand breaks, inflammation, and markers of oxidative DNA damage [26].

5. Conclusion

In the context of the present limited information, it is stated that DNA damage may be high in patients diagnosed with depression and anxiety disorders and receiving medical treatment. Our study has concluded that in patients with depression and anxiety disorders under treatment, single or double-stranded DNA breaks and DNA damage in peripheral lymphocytes were higher, independent from age, sex, and the type of

treatment drug (SSRIs or SNRIs). For this reason, inappropriate or excessive use of psychiatric drugs may represent potential health risks. Therefore, dosage and duration of treatment should be given ultimate attention. The results of our study contributed to revealing the possible genotoxic potential of depression and anxiety disease or the drugs used in their treatment. However, it is important to quantify the study findings with other test methods commonly used to determine their genotoxicity.

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Table 1. The drugs and dosages of drugs in the patient group

Drug name	Number of patients	Drug name	Dosage (mg)/ day	
			Min	Max
Duloxetine	4	Aripiprazole	30	60
Duloxetine + Aripiprazole	1		10	10
Escitalopram	2	Trazodone	10	15
Escitalopram + Trazodone	1	Mirtazapine	50	50
Escitalopram + Mirtazapine	1	Trifluperazine	30	30
Escitalopram + Trifluperazine	2		1	2
Fluoxetine	6	Vortioksetin	20	60
Fluoxetine + Vortioksetin	1	Aripiprazole	5	5
Fluoxetine + Aripiprazole	2	Trazodone	5	5
Fluoxetine + Trazodone	1	Trifluperazine	50	50
Fluoxetine + Trifluperazine	2	Mirtazapine	1	2
Fluoxetine + Mirtazapine	2		15	15
Quetiapine + Bupropion	1	Bupropion	200	300
Paroksetin + Aripiprazole	1	Aripiprazole	20	20
Sertaline	5	Buspirone	50	100
Sertaline + Buspirone	1	Trifluperazine	15	15
Sertaline + Trifluperazine	2		1	1
Venlafaxine	2	Trifluperazine	75	150
Venlafaxine + Trifluperazine	1		1	1

Table 2. Level of DNA damage in patient groups and control group in the comet assay

Group	N	Tail length (µm)		Tail intensity (%)		Tail Moment	
		TL ± SE	Median	TI ± SE	Median	TM±SE	Median
PG	38	107,4±9,46	81,45 [a]	25,72±1,58	26,18 [a]	14,55±1,37	12,36 [a]
CG	28	72,04±7,51	59,74	15,86±1,80	13,43	6,23±0,93	4,32
PG (20-35 Age)	13	95,76±9,87	83,86	25,03±2,54	24,59	12,92±1,69	12,19
PG (36-50 Age)	25	113,44±13,42	77,22	26,07±2,03	27,73	15,39±1,9	13,42
PG Female	27	102,95±10,64	80,64	25,45 ±1,89	27,16	14,0±1,53	12,4
PG Male	11	118,31±20,14	82,27	26,36 ±3,0	25,20	15,9 ± 3,0	12,19
Initial diagnosis	21	89,28 ±9,65	72,21	25,37 ±2,13	27,73	13,04 ±1,63	11,98
Recurrent diagnosis	17	129,77±16,21	140,66	26,14 ±2,41	25,2	16,4 ±2,3	12,4
SSRI	29	109,88±11,14	82,27	26,29 ±1,81	24,59	15,3 ±1,66	12,33
SNRI	8	99,37 ±18,41	77,22	23,85 ±3,33	27,73	12,11 ±2,21	12,4
Monotherapy	19	123,20 ±16,6	88,10	28,4 ±2,57	30,82	17,51 ±2,29	14,10 [b]
Combined therapy	19	91,59 ±8,05	77,22	23,03 ±1,67	23,10	11,59 ±1,25	11,94
Fluoxetine monotherapy	6	129 ±35,72	84,37	31,54 ±4,87	35,94	22,34 ±4,75	24,12
Single drug treatment (except fluoxetine)	13	120,48±18,9	92,57	26,96 ±3,06	28,75	15,28 ±2,41	13,42
Combine fluoxetine	8	102,89±14,84	90,09	23,55 ±2,5	23,84	11,09 ±1,68	11,30
Combined treatment other than fluoxetine	11	83,37 ±8,57	74,31	22,65 ±2,34	23,09	11,95 ±1,84	11,94
Fluoxetine	14	114,12±17,06	85,19	26,97 ±2,65	27,28	15,91 ±2,64	12,67
Patient not under fluoxetine treatment	24	103,47±11,42	75,76	24,98 ±1,99	26,28	13,75 ±1,56	12,36

Mann-Whitney U test.
For Comet assay, 200 lymphocytes were scored for each donor.
[a] Significantly different from the control group at $p < 0.01$.
[b] Significantly different from the control group at $p < 0.05$.
Abbreviations; N: Number of subjects; TL: Tail length; TI: Tail intensity; TM: Tail Moment; PG: Patient group; CG: Control group; Fluoxetine: Fluoxetine (mono + combined) treatment group.

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