Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1691200

# Does the level of oxidative stress and genotoxicity increase in patients with acute myeloid leukemia?

*Akut miyeloid lösemi hastalarında oksidatif stres ve genotoksisite düzeyi artıyor mu?*

Gülsüm Akgün Çağlıyan, Fatih Altıntaş, Başak Ünver Koluman, Melek Tunç Ata, Mehmet Nuri Başer, Emine Kılıç Toprak, Ayşegül Çört, Melek Bor Küçükatay

Posted date:04.05.2025 Acceptance date:16.06.2025

**Abstract**

**Purpose:** The present study evaluates the levels of oxidative stress and DNA damage in patients diagnosed with acute myeloid leukemia (AML; newly diagnosed patients and patients in remission) compared to healthy controls.

**Materials and methods:** A total of 96 participants were enrolled into this study in three groups: 32 newly diagnosed AML patients, 32 patients in remission, and 32 age- and sex-matched healthy individuals. Various laboratory analyses were conducted to measure biochemical parameters, total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and DNA damage using the comet assay.

**Results:** We showed that while TOS levels did not differ significantly between the groups, both the newly diagnosed and remission groups exhibited higher OSI levels compared to controls. TAS levels were notably lower in the remission group, suggesting decreased antioxidant capacity. The comet assay demonstrated significantly elevated DNA damage in the newly diagnosed AML group, followed by the remission group, relative to healthy controls. These findings highlight an increased oxidative stress burden and genotoxicity in AML patients, which may contribute to disease pathophysiology. Furthermore, the results suggest that the oxidative stress and DNA damage are more pronounced in newly diagnosed patients compared to those in remission.

**Conclusion:** The study showed that the oxidative stress and DNA damage observed in AML patients, particularly at diagnosis, could play a role in the progression and recurrence of the disease, suggesting potential prognostic value for oxidative stress markers in AML management.

**Keywords:** Oxidative stress, acute myeloid leukemia, genotoxicity.

Akgun Cagliyan G, Altintas F, Unver Koluman B, Tunc Ata M, Baser MN, Kilic Toprak E, Cort A, Bor Kucukatay M. Does the level of oxidative stress and genotoxicity increase in patients with acute myeloid leukemia? Pam Med J 2025;18:749-759.

**Öz**

**Amaç:** Bu çalışmada akut myeloid lösemi tanılı hastalarda (AML; yeni tanı ve remisyondaki hastalar) oksidatif stres ve DNA hasarı düzeyleri sağlıklı kontrollerle karşılaştırılarak değerlendirilmiştir.

**Gereç ve yöntem:** Bu çalışmaya üç grup toplam, 96 katılımcı; 32 yeni AML hastası, 32 remisyondaki hasta ve 32 yaş ve cinsiyete uygun sağlıklı birey dahil edildi. Biyokimyasal parametreleri, toplam oksidan durumu (TOS), toplam antioksidan durumu (TAS), oksidatif stres indeksi (OSI) ve DNA hasarını ölçmek için comet testi kullanılarak çeşitli laboratuvar analizleri yapıldı.

**Bulgular:** TOS seviyelerinin gruplar arasında önemli ölçüde farklılık göstermediğini gösterdik, ancak hem yeni tanı hem de remisyon grupları kontrollerle karşılaştırıldığında daha yüksek OSI seviyeleri sergiledi. TAS seviyeleri remisyon grubunda belirgin şekilde daha düşüktü ve bu da azalmış antioksidan kapasitesini gösteriyordu.

Comet testi, sağlıklı kontrollere kıyasla yeni tanı AML grubunda, ardından remisyon grubunda önemli ölçüde yüksek DNA hasarı gösterdi.

Gülsüm Akgün Çağlıyan, Assoc. Prof. University of Pamukkale School of Medicine Department of Hematology, Denizli, Türkiye, e-mail: drgulsumakgun@gmail.com (https://orcid.org/0000-0002-2073-1949) (Corresponding Author)

Fatih Altıntaş, PhD. University of Pamukkale School of Medicine Department of Physiology, Denizli, Türkiye, e-mail: faltintas@pau.edu.tr (https://orcid.org/0000-0001-8779-0110)

Başak Ünver Koluman, Asst. Prof. University of Pamukkale School of Medicine Department of Hematology, Denizli, Türkiye, e-mail: bkoluman@pau.edu.tr (https://orcid.org/0000-0003-1106-5021)

Melek Tunç Ata, PhD. University of Pamukkale School of Medicine Department of Physiology, Denizli, Türkiye, e-mail: melekt@pau.edu.tr (https://orcid.org/0000-0002-0384-2356)

Mehmet Nuri Başer, PhD. University of Adnan Menderes School of Medicine Department of Medical Oncology, Aydın, Türkiye, e-mail: mehmetnuribaser@gmail.com (https://orcid.org/0000-0003-1809-5581)

Emine Kılıç Toprak, Assoc. Prof. University of Pamukkale School of Medicine Department of Physiology, Denizli, Türkiye, e-mail: ektoprak@pau.edu.tr (https://orcid.org/0000-0002-8795-0185)

Ayşegül Çört, Prof. University of Pamukkale School of Medicine Department of Biochemistryi, Denizli, Türkiye, e-mail: aysegulcort@gmail.com (https://orcid.org/0000-0001-8946-7173)

Melek Bor Küçükatay, Prof. University of Pamukkale School of Medicine Department of Physiology, Denizli, Türkiye, e-mail: mbor@pau.edu.tr (https://orcid.org/0000-0002-9366-0205)

Bu bulgular, AML hastalarında hastalığın patofizyolojisine katkıda bulunabilecek artmış oksidatif stres yükü ve genotoksisiteyi vurgulamaktadır. Dahası, sonuçlar oksidatif stres ve DNA hasarının remisyondaki hastalara kıyasla yeni tanı hastalarda daha belirgin olduğunu göstermektedir.
**Sonuç:** Çalışmamızda, AML hastalarında özellikle tanı anında gözlenen oksidatif stres ve DNA hasarının hastalığın ilerlemesinde ve tekrarlamasında rol oynayabileceği gösterilmiş olup, AML yönetiminde oksidatif stres belirteçlerinin potansiyel prognostik değere sahip olabileceği düşünülmektedir.

**Anahtar kelimeler:** Oksidatif stres, akut miyeloid lösemi, genotoksisite.

Akgün Çağlıyan G, Altıntaş F, Ünver Koluman B, Tunç Ata M, Başer MN, Kılıç Toprak E, Çört A, Bor Küçükatay M. Akut miyeloid lösemi hastalarında oksidatif stres ve genotoksisite düzeyi artıyor mu? Pam Tıp Derg 2025;18:749-759.

## Introduction

Acute myeloid leukemia (AML) is a heterogeneous blood cancer defined by the abnormal growth of myeloid blasts in the peripheral blood, bone marrow, and other organs [1]. The prevalence of frequent among adults, with an incidence rate of 3.6 per 100,000 individuals [2]. The median age at diagnosis is 68 years, with 70% of newly diagnosed cases occurring in individuals aged 55 years and older [3]. AML can be classified into several subtypes, each having different methods of treatment. Acute promyelocytic leukemia (AML M3) represents 10% of all instances of acute myeloid leukemia (AML) and exhibits distinct pathophysiology, clinical, and therapeutic features. Despite its favorable prognosis, the presence of comorbidities such as coagulopathy, and thrombocytopenia increases the chance of early mortality by 20-30%. After the use of all-trans retinoic acid clinically, AML M3 becomes the most curable subtype of AML with a cure rate of 80%. However, for patients with non-AML M3, the 5-year overall survival is approximately 45% in young patients (≤60 years old), whereas it is less than 10% in old patients (>60 years old) [4]. Biological fluids may include varying concentrations of oxidant chemicals. An imbalance between these molecules, called reactive oxygen species, and antioxidants can trigger oxidative stress, leading to oxidative damage to DNA, lipids and proteins and thus promoting the growth of tumor cells [5]. The stress caused by ROS is associated with the development of different types of malignancies, including AML. Leukemia stem cells (LSC) are effectively eliminated by high levels of reactive oxygen species (ROS), but the task of minimizing the harmful effects of ROS on normal cells is a new obstacle in oxidative stress therapy for leukemia [6]. DNA damage and defects in DNA repair are causally related to cancer, aging and genetic diseases [7]. The aim of this study was to examine the systemic parameters of oxidative stress and levels of DNA damage in patients with newly diagnosed AML and in patients with AML in remission; compared to a control group consisting of people matched in terms of age and sex who are in healthy condition. An examination of DNA damage and levels of oxidant-antioxidant in these patients may associate and contribute to the basic pathophysiology of AML and it would benefit patients in terms of the investigation of new therapeutic agents.

## Patients and methods

Permission was obtained from Pamukkale University, Non-Interventional Clinical Research Ethics Committee for the study (approval number 60116787-020/59517 at the meeting dated 29/09/2020 and numbered 18). The participants were informed about the study and provided verbal and written consent. This study is in accordance with the ethical standards of the institutional and/or national research committees and the Declaration of Helsinki.

### Groups

The study included 32 newly diagnosed AML patients (Group 1), 32 patients with AML in remission (Group 2) and 32 age- and sex-matched healthy controls (Group 3). Diagnosis was confirmed using peripheral smear, bone marrow aspiration/biopsy and flow cytometry based on WHO criteria published somewhere else. The treatment of all patients with AML was performed according to current national and international clinical guidelines and current rules.

**Laboratory analysis**

Venous blood samples were collected from all participants at a volume of approximately 15 ml. A complete blood count was conducted on the blood samples. Biochemical tests were also conducted, including those for urea, creatinine, AST, ALT, LDH, uric acid, total protein, albumin, sodium, potassium, calcium, and phosphorus. Additionally, coagulation tests were performed, including prothrombin time, activated partial thromboplastin time, fibrinogen, and d-dimer.

**Total oxidant status (TOS) and total antioxidant status (TAS) measurement**

Following centrifugation of the blood samples, the serum was stored at -80°C until the study day, at which measurements of total oxidizable substances (TOS) and total antioxidant status (TAS) were conducted. TOS and TAS were quantified from serum samples using commercially available kits (Rel Assay Diagnostic, Türkiye). TOS and TAS measurements were determined spectrophotometrically with an ELISA reader at 492 nm and 405 nm wavelengths, respectively. The results were expressed per µmol of hydrogen peroxide equivalent and µmol of Trolox equivalent. These values were expressed per mg of protein, respectively.

**Oxidative stress index (OSI) calculation**

An additional indicator of the level of oxidative stress is the oxidative stress index (OSI), which is obtained through calculation. The index was calculated using the following formula, which employs the values of TOS and TAS:

The oxidative stress index (OSI) is calculated using the following formula:

OSI = TOS (µmol $H_2O_2$ eqv/L) / TAS (µmol Trolox eqv/L) x 100.

**DNA damage analysis by comet assay**

Single-cell gel electrophoresis, or comet analysis, is a highly sensitive, reliable, reproducible, simple and rapid method for determining DNA damage [8]. It is typically employed by isolating peripheral lymphocytes. A total of five milliliters of anticoagulated blood was obtained from the experimental groups and transferred into a tube. This was then diluted in a 1:1 ratio with phosphate buffer (PBS). In a separate tube, 3 ml of Ficol-1077 was added, and 10 ml of the diluted blood sample was subsequently transferred. The sample was then subjected to centrifugation at 400 ×g for a period of 20 minutes. Subsequently, the lymphocytes situated within the nebular layer, located at the center of the tube, were isolated. Following two or three washes with PBS, the cells were counted using a Neubauer slide and adjusted to 1 x $10^5$ cells per 100 microliters. The samples were stored at -80°C until analysis by comet assay. On the day of the experiment, 20 microliters of the lymphocyte suspension were taken and resuspended with 60 microliters of low melting agarose (LMA), prepared at 37°C. The mixture of LMA and cells was applied as a thin layer to a slide that had been previously coated with 1% normal melting agarose. Following a 15-minute incubation period at 4°C, the slide was covered with a third layer of 70 µl of 0.5% LMA and maintained on ice for a further 15 minutes. Subsequently, the slide was treated with cold lysis buffer with a pH of 10 at +4°C for 60 minutes to remove cellular membranes. Subsequently, the slides were transferred to a horizontal gel electrophoresis tank and incubated in freshly prepared alkaline electrophoresis buffer for 30 minutes. Subsequently, the slides were subjected to electrophoresis at 4°C under a 300-mA current for 30 minutes. Subsequently, the slides were washed on three occasions for a period of five minutes with cold neutralization buffer (0.4M Tris-HCl, pH 7.5) to remove any remaining alkaline substances and detergents. Following neutralization, the slides were stained with 60 µl ethidium bromide (2 µl/ml) and examined under a fluorescence microscope. The possible DNA damage was then evaluated with the “Comet assay IV system (AutoComet)” software. The following parameters were employed: head length (HL, µm), tail length (TL, µm), head density (percentage of DNA in the head, expressed as % H-DNA), tail density (percentage of DNA in the tail, % T-DNA), tail moment (TM, expressed in µm, is the product of % T-DNA and TL divided by 100), and tail migration (the length of DNA migration from the edge of the head to the smallest detectable fragment).

### Serum 8-hydroxy-deoxyguanosine (8-OHdG) measurement

Reactive oxygen species have been demonstrated to be a primary catalyst in the formation of over 20 distinct oxidative base damage products within the context of DNA. Among these damaged bases, 8-OHdG is the most sensitive and most prevalent oxidative DNA damage marker and can be quantified in serum. The blood samples were analyzed spectrophotometrically with a commercial kit using an ELISA reader at a wavelength of 450 nm, in accordance with the kit protocol.

### Statistical analysis

The statistical analysis was conducted using the SPSS 28.0 for Windows software (IBM Corp., Armonk, New York, United States). In this study, the aim was to compare oxidative stress index (OSI) levels among three independent groups: newly diagnosed AML patients, AML patients in remission, and healthy controls. A total of 96 participants were included in the analysis, with 32 individuals in each group. A post hoc power analysis was conducted based on the differences in group means, assuming a medium effect size (f=0.35). The significance level was set at 5% (α=0.05). Under these conditions, the statistical power of the study was calculated to be 84.6% (power=0.846). This result indicates a high probability of detecting a statistically significant difference among the groups. Descriptive statistics were presented as numbers and percentages for categorical variables and as means, standard deviations, minimums, maximums, and medians for numerical variables. The observed differences in the proportions of the groups were evaluated using the chi-square test. Comparisons of numerical variables were conducted using the one-way ANOVA test when the conditions for normal distribution were met and the Kruskal-Wallis test when these conditions were not met. Subgroup analyses were conducted using the non-parametric Mann-Whitney U test, with Bonferroni correction applied for interpretation. This was performed for data sets comprising more than two groups. As the normal distribution condition was not met, the relationships between the numerical data were analyzed by means of a Spearman correlation analysis. The alpha significance level was set at $p<0.05$.

### Results

All the demographic data (age, gender, and body mass index) of the participants in the groups are similar. The relapse rate of Group 1 (newly diagnosed AML) within one year was found to be statistically significantly higher than that of Group 2 (remission AML) ($p=0.024$). Furthermore, a statistically significant difference was observed in the death rates of the groups ($p<0.001$). The mortality rate of Group 1 was higher than that of Group 2 (control) (Table 1a and Table 1b).

**Table 1a.** Demographic characteristics of AML patients and control

| | | Group 1 (n=32) (New diagnosed AML) | Group 2 (n=32) (Remission in AML) | Group 3 (n=32) (Control) | *P* (Test statistic) |
|---|---|---|---|---|---|
| **Age Mean±SD** | | 66.2±12.7 | 61.5±16.3 | 67.6±11.2 | 0.372‡ (1.980) |
| **Min-Max (Median)** | | 35-88 (70) | 18-82 (65) | 46-86 (67.5) | |
| **Gender n (%)** | **Female** | 12 (37.5) | 18 (56.25) | 18 (52.9) | 0.276# (2.578) |
| | **Male** | 20 (62.5) | 14 (43.75) | 16 (47.1) | |
| **BMI** | | | | | |
| Mean±SD | | 27.2±4.3 | 26.7±3.6 | 25.2±4.0 | 0.110† (2.255) |
| Min-Max (Median) | | 19.3-37.1 (26.7) | 20.1-35.7 (26.5) | 17-34.5 (25) | |

BMI: Body Mass Index, † One Way ANOVA test, ‡Kruskal–Wallis test, # Chi Square test

**Table 1b.** Demographic characteristics of AML patients

| | | Group 1 (n=32) (New diagnosed AML) | Group 2 (n=32) (Remission in AML) | *P* (Test statistic) |
|---|---|---|---|---|
| **AML n (%)** | NON-M3 | 30 (93.8) | 30 (93.8) | 1.000# (0.000) |
| | M3 | 2 (6.3) | 2 (6.3) | |
| **FAB n (%)** | M0 | 1 (3.1) | 0 (0.0) | 0.769# (3.187) |
| | M1 | 2 (6.3) | 0 (0.0) | |
| | M2 | 9 (28.1) | 10 (31.3) | |
| | M3 | 2 (6.3) | 2 (6.3) | |
| | M4 | 4 (12.5) | 6 (18.8) | |
| | M5 | 14 (43.8) | 14 (43.8) | |
| **Genetics n (%)** | Good | 9 (28.1) | 10 (31.3) | 0.864# (0.293) |
| | Medium | 12 (37.5) | 13 (40.6) | |
| | High Risk | 11 (34.4) | 9 (28.1) | |
| **Within 1 Year Relapses n (%)** | None | 12 (37.5) | 21 (65.6) | 0.024#* (5.067) |
| | Yes | 20 (62.5) | 11 (34.4) | |
| **Last Status n (%)** | Alive | 8 (25.0) | 19 (59.4) | <0.001#* (39.624) |
| | Exitus | 24 (75.0) | 13 (40.6) | |

AML: Acute Myeloid Leukemia, FAB: French–American–British classification, # Chi Square test, *$p<0.05$

A statistically significant difference was observed in the biochemical laboratory characteristics of the participants, except for the AST and phosphorus levels (AST $p=0.873$, phosphorus $p=0.401$; all other comparisons $p<0.05$). The sodium and potassium levels were found to be statistically significantly lower, while the uric acid and creatinine levels were observed to be higher in Group 1 when compared to Group 2 ($p=0.031$, $p=0.005$, $p=0.032$, $p=0.006$). The total protein, calcium and ALT levels were found to be statistically significantly lower in Group 1 compared to both Group 2 and Group 3 ($p=0.025$, $p=0.001$ and $p=0.001$, respectively). Additionally, the D-dimer and PT levels were observed to be statistically significantly higher in Group 1 compared to both Group 2 and Group 3 ($p=0.001$, $p=0.011$ and $p<0.001$, respectively). The levels of urea, APTT and fibrinogen were found to be significantly higher in Group 1 and Group 2 in comparison to Group 3 ($p<0.001$, $p=0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.001$, respectively). The albumin level in Group 1 was found to be lower than that observed in Group 2 and Group 3. Furthermore, the albumin level in Group 2 was found to be statistically significantly lower than that observed in Group 3 ($p=0.003$, $p<0.001$, $p<0.001$, $p=0.013$) (Table 2a and 2b).

No statistically significant difference was observed between the TOS levels of the patient and control groups. However, there were statistically significant differences in the TAS and OSI levels of the groups ($p=0.007$ and $p=0.008$, respectively). The TAS level of Group 2 was found to be statistically significantly lower than that of Group 3 ($p=0.003$). Additionally, the OSI level of both Group 1 and Group 2 was observed to be statistically significantly higher than that of Group 3 ($p=0.006$ and $p=0.010$, respectively) (Table 3a and 3b). The results indicate that the serum oxidative stress level is elevated in patients with AML.

**Table 2a.** Biochemical laboratory characteristics of AML patients and control

| | Group 1 (New diagnosed AML) | Group 2 (Remission in AML) | Group 3 (Control) | *P* (Test statistic) |
|---|---|---|---|---|
| | Mean±SD<br>Min-Max (Median) | Mean±SD<br>Min-Max (Median) | Mean±SD<br>Min-Max (Median) | |
| **Urea** | 46.5±29.5<br>15-131 (34.5) | 38.5±25.7<br>16-138 (30.5) | 25.6±6.3<br>14-44 (24) | <0.001‡* (19.107) |
| **Creatinine** | 1.18±1.00<br>0.37-6.04 (0.92) | 0.90±0.41<br>0.36-2.49 (0.82) | 0.77±0.17<br>0.42-1.34 (0.75) | 0.025‡* (7.392) |
| **Sodium** | 138.0±4.9<br>129-152 (138) | 139.6±2.4<br>135-144 (140) | 140.2±2.2<br>136-146 (140) | 0.034†* (3.510) |
| **Potassium** | 4.05±0.63<br>2.94-5.49 (4.06) | 4.19±0.58<br>3.14-5.75 (4.21) | 4.49±0.42<br>3.81-5.49 (4.44) | 0.005†* (5.528) |
| **Total Protein** | 61.7±9.9<br>42.1-81.98 (64.09) | 66.9±8.1<br>51.6-81.9 (68.9) | 69.2±4.5<br>60.7-78.89 (68.63) | 0.001†* (7.870) |
| **Albumin** | 34.9±7.8<br>20.7-47.43 (36.635) | 40.8±6.8<br>26.3-56.7 (42.4) | 44.4±2.9<br>38.5-49.44 (44.64) | <0.001‡* (29.507) |
| **AST** | 22.6±22.1<br>5-120 (16.5) | 17.8±8.9<br>7-49 (16) | 16.2±4.0<br>10-28 (15) | 0.873‡ (0.272) |
| **ALT** | 14.9±11.2<br>2-53 (11) | 19.6±12.8<br>5-72 (16) | 18.4±9.2<br>6-47 (16) | 0.013‡* (8.619) |
| **LDH** | 619.4±817.9<br>127-4436 (348.5) | 195.5±66.8<br>99-391 (187) | 172.5±30.8<br>125-277 (163) | <0.001‡* (28.891) |
| **Uric acid** | 5.95±3.47<br>1.2-15.7 (5.65) | 4.62±1.67<br>0.80-8.40 (4.80) | 4.50±1.14<br>2.10-7.30 (4.35) | 0.022†* (3.980) |
| **Calcium** | 8.49±0.90<br>6.18-10.16 (8.63) | 9.12±0.59<br>7.64-10.46 (9.11) | 9.25±0.40<br>8.42-10.16 (9.29) | <0.001†* (12.278) |
| **Phosphor** | 3.39±1.28<br>0.99-8.61 (3.29) | 3.51±0.51<br>1.99-4.48 (3.54) | 3.40±0.53<br>2.51-4.47 (3.42) | 0.401‡ (1.827) |
| **APTT** | 29.1±4.2<br>21.6-38.8 (28.75) | 30.2±3.7<br>23.9-41.5 (29.4) | 25.5±2.5<br>22.1-35.5 (25.1) | <0.001‡* (30.035) |
| **D-dimer** | 2557.8±3386.3<br>151-13736 (785) | 314.2±279.0<br>51-1154 (237.5) | 325.9±136.7<br>190-680 (300) | <0.001‡* (29.560) |
| **Fibrinogen** | 404.9±161.7<br>129-747 (423) | 354.3±97.1<br>207-589 (342.5) | 274.2±82.9<br>171-447 (250) | <0.001‡* (16.389) |
| **PT** | 15.2±3.9<br>10.5-30.8 (14.2) | 12.5±2.9<br>10.2-27 (11.9) | 11.7±0.7<br>10.2-13.5 (11.65) | <0.001‡* (33.333) |

AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, LDH: Lactate Dehydrogenase, APTT: Activated Partial Thromboplastin Time PT: Prothrombin Time, †One Way ANOVA, ‡Kruskal–Wallis test, * $p<0.05$

**Table 2b.** Subgroup analyses

| | | | *p* | | P£ | | p£ |
|---|---|---|---|---|---|---|---|
| **Group 1 vs.** | **Group 2** | | 0.160 | | 0.160 | | 0.248 |
| | **Group 3** | **Sodium** | 0.031* | **Urea** | <0.001* | **Phosphor** | 0.555 |
| **Group 2 vs.** | **Group 3** | | 0.765 | | 0.001* | | 0.287 |
| **Group 1 vs.** | **Group 2** | | 0.569 | | 0.158 | | 0.298 |
| | **Group 3** | **Potassium** | 0.005* | **Creatinine** | 0.006* | **APTT** | <0.001* |
| **Group 2 vs.** | **Group 3** | | 0.074 | | 0.223 | | <0.001* |
| **Group 1 vs.** | **Group 2** | | 0.025* | | 0003* | | <0.001* |
| | **Group 3** | **Total Protein** | 0.001* | **Albumin** | <0.001* | **D-dimer** | <0.001* |
| **Group 2 vs.** | **Group 3** | | 0.466 | | 0.013* | | 0.061 |
| **Group 1 vs.** | **Group 2** | | 0.057 | | 0.726 | | 0.212 |
| | **Group 3** | **Uric acid** | 0.032* | **AST** | 0.738 | **Fibrinogen** | <0.001* |
| **Group 2 vs.** | **Group 3** | | 0.978 | | 0.605 | | 0.001* |
| **Group 1 vs.** | **Group 2** | | 0.001* | | 0.011* | | <0.001* |
| | **Group 3** | **Calcium** | <0.001* | **ALT** | 0.011* | **PT** | <0.001* |
| **Group 2 vs.** | **Group 3** | | 0.701 | | 0.893 | | 0.422 |

AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, APTT: Activated Partial Thromboplastin Time, PT: Prothrombin Time £Bonferroni Correction, $p<0.017$, *$p<0.05$

**Table 3a.** TOS, TAS and OSI values between groups

| | **Group 1 (n=32) (New diagnosed AML)** | **Group 2 (n=32) (Remission in AML)** | **Group 3 (n=32) (Control)** | ***P* (Test statistic)** |
|---|---|---|---|---|
| | **Mean±SD Min-Max (Median)** | **Mean±SD Min-Max (Median)** | **Mean±SD Min-Max (Median)** | |
| **TOS (µmol/L)** | 6.27±2.18<br>3.57-12.94 (5.93) | 5.97±2.82<br>1.65-12.86 (5.66) | 4.72±3.29<br>0.62-12.62 (5.32) | 0.234‡<br>(2.910) |
| **TAS (µmol/L)** | 1.36±0.49<br>0.62-2.62 (1.26) | 1.28±0.32<br>0.78-2.20 (1.25) | 1.56±0.39<br>0.48-1.99 (1.66) | 0.007‡*<br>(9.792) |
| **OSI** | 513.8±253.2<br>226.8-1191.8 (433.7) | 508.1±291.3<br>84.2-1212.3 (455.4) | 371.6±460.2<br>40.4-2640.4 (336.6) | 0.008‡*<br>(9.663) |

TAS: Total Antioxidant Status, TOS: Total Oxidant Status, OSI: Oxidative Stress Index, ‡Kruskal–Wallis test, *$p<0.05$

**Table 3b.** Subgroup analyses

| | | | p£ | | P£ | | p£ |
|---|---|---|---|---|---|---|---|
| **Group 1 vs.** | **Group 2** | | 0.379 | | 0.683 | | 0.811 |
| | **Group 3** | **TOS (µmol/L)** | 0.191 | **TAS µmol/L)** | 0.020* | **OSI** | 0.006* |
| **Group 2 vs.** | **Group 3** | | 0.135 | | 0.003* | | 0.010* |

£Bonferroni Correction, $p<0.017$, *$p<0.05$

No statistically significant differences were observed between the groups in serum 8-OHdG levels, a marker of oxidative DNA damage ($p$=0.508) (Table 4). The results indicate that oxidative stress does not appear to be a primary cause of DNA damage in AML patients.

The comet experiment was performed in our study. Statistically significant difference was observed between the groups in terms of tail length, head intensity, tail intensity, tail moment, and tail migration levels, which provide insight into DNA damage ($p$<0.001 for all). The tail length, tail moment, and tail migration levels of Group 1 were found to be statistically significantly higher than those of Group 2 and Group 3 ($p$<0.001 for all). The head intensity level of Group 1 was found to be significantly lower than that of Group 2 and Group 3, while the head intensity level of Group 2 was also found to be significantly lower than that of Group 3 ($p$=0.007, $p$<0.001, $p$<0.001, $p$<0.001, respectively). The tail intensity level of Group 1 was found to be significantly higher than that of Groups 2 and 3, while the tail intensity level of Group 2 was also found to be significantly higher than that of Group 3 ($p$=0.007, $p$<0.001, $p$<0.001) (Tables 5a and 5b). These findings unambiguously demonstrate the occurrence of genotoxicity in the lymphocytes of patients with AML.

**Table 4.** Serum 8-OHdG levels of the groups

| | **Group 1 (New diagnosed AML)** | **Group 2 (Remission in AML)** | **Group 3 (Control)** | ***P* (Test statistic)** |
|---|---|---|---|---|
| | **Mean±SD Min-Max (Median)** | **Mean±SD Min-Max (Median)** | **Mean±SD Min-Max (Median)** | |
| **8-OHdG** | 14.0±21.2<br>0.5-85.9 (5.7) | 9.3±7.5<br>1.7-38.3 (6.3) | 9.1±5.6<br>2.7-30.6 (8.7) | 0.508‡<br>(1.354) |

8-OHdG: 8-hidroksi-2' -deoksiguanozin, ‡Kruskal–Wallis test

**Table 5a.** Head length, tail length, head intensity, tail intensity, tail moment, tail migration levels of the groups

| | **Group 1 (New diagnosed AML)** | **Group 2 (Remission in AML)** | **Group 3 (Control)** | ***P* (Test statistic)** |
|---|---|---|---|---|
| | **Mean±SD Min-Max (Median)** | **Mean±SD Min-Max (Median)** | **Mean±SD Min-Max (Median)** | |
| **Head Length** | 30.3±3.4<br>22.7-38.3 (29.6) | 30.0±3.2<br>22.6-39.3 (30.1) | 29.0±2.3<br>24.5-34.4 (28.4) | 0.170†<br>(1.805) |
| **Tail Length** | 39.8±9.4<br>21.9-63.9 (39.8) | 34.8±8.9<br>22.2-54.9 (33.1) | 24.0±6.2<br>16.1-46.1 (22.2) | <0.001‡*<br>(41.251) |
| **Head Intensity** | 54.6±17.2<br>16.6-89 (51.5) | 66.4±17.1<br>22.1-89.5 (70.9) | 83.5±9.4<br>49.5-94.1 (86.1) | <0.001‡*<br>(40.978) |
| **Tail Intensity** | 45.4±17.2<br>11-83.4 (48.5) | 33.6±17.1<br>10.5-77.9 (29.1) | 16.5±9.4<br>5.9-50.5 (13.9) | <0.001‡*<br>(40.978) |
| **Tail Moment** | 9.43±5.00<br>1.16-24.8 (9.53) | 6.67±4.51<br>1.29-19.9 (5.78) | 2.52±2.07<br>0.59-10.68 (1.81) | <0.001‡*<br>(39.875) |
| **Tail Migration** | 24.7±10.0<br>7.7-52.6 (25) | 19.8±9.4<br>6.1-43.6 (17.4) | 9.5±6.1<br>3.6-32.7 (7.2) | <0.001‡*<br>(40.922) |

† One Way ANOVA test, ‡Kruskal–Wallis test, *$p$<0.05

**Table 5b.** Subgroup analyses

| | | | p£ | | P£ | | p£ |
|---|---|---|---|---|---|---|---|
| **Group 1 vs.** | **Group 2** | **Head Length** | 0.862 | **Tail Length** | 0.055 | **Head Intensity** | 0.007* |
| | **Group 3** | | 0.080 | | <0.001* | | <0.001* |
| **Group 2 vs.** | **Group 3** | | 0.054 | | <0.001* | | <0.001* |
| **Group 1 vs.** | **Group 2** | **Tail Intensity** | 0.007* | **Tail Moment** | 0.020 | **Tail Migration** | 0.046* |
| | **Group 3** | | <0.001* | | <0.001* | | <0.001* |
| **Group 2 vs.** | **Group 3** | | <0.001* | | <0.001* | | <0.001* |

£Bonferroni Correction, $p<0.017$, *$p<0.05$

A negative and statistically significant strong correlation was observed between OSI level and uric acid level in group 1 ($p<0.001$). In group 2, the OSI level was observed to exhibit a positive and statistically significant weak correlation with sodium ($p=0.047$). In group 3, a weak positive correlation was observed between tail length and urea, while a weak negative correlation was noted between tail length and APTT, with statistical significance ($p=0.032$, $p=0.024$) (Table 6).

**Table 6.** OSI and tail length correlation of biochemical parameter analyses

| | **Group 1 (New diagnosed AML)** | | | | **Group 2 (Remission in AML)** | | | | **Group 3 (Control)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OSI** | | **Tail Length** | | **OSI** | | **Tail Length** | | **OSI** | | **Tail Length** | |
| | **r** | ***p*** | **r** | ***p*** | **r** | ***p*** | **r** | ***p*** | **r** | ***p*** | **r** | ***p*** |
| **Urea** | -0.297 | 0.099 | 0.143 | 0.458 | -0.172 | 0.364 | -0.109 | 0.552 | 0.029 | 0.870 | 0.368 | 0.032* |
| **Creatinine** | -0.332 | 0.063 | -0.019 | 0.920 | -0.315 | 0.090 | 0.016 | 0.931 | -0.193 | 0.275 | 0.324 | 0.061 |
| **Sodium** | 0.088 | 0.632 | 0.146 | 0.449 | 0.365 | 0.047* | -0.134 | 0.466 | 0.162 | 0.359 | -0.019 | 0.913 |
| **Potassium** | -0.263 | 0.146 | 0.084 | 0.664 | 0.172 | 0.365 | -0.158 | 0.388 | -0.049 | 0.783 | 0.179 | 0.311 |
| **Total Protein** | -0.135 | 0.460 | 0.099 | 0.609 | 0.064 | 0.736 | -0.011 | 0.954 | -0.217 | 0.217 | 0.236 | 0.179 |
| **Albumin** | -0.040 | 0.828 | 0.343 | 0.069 | 0.116 | 0.541 | -0.044 | 0.810 | 0.029 | 0.873 | -0.001 | 0.995 |
| **AST** | -0.313 | 0.081 | -0.058 | 0.764 | 0.047 | 0.806 | 0.290 | 0.108 | 0.132 | 0.457 | 0.080 | 0.652 |
| **ALT** | -0.004 | 0.982 | 0.124 | 0.520 | -0.036 | 0.852 | 0.117 | 0.524 | 0.230 | 0.191 | 0.216 | 0.219 |
| **LDH** | -0.320 | 0.075 | -0.024 | 0.903 | -0.345 | 0.062 | 0.208 | 0.253 | 0.099 | 0.576 | -0.195 | 0.268 |
| **Uric acid** | -0.739 | <0.001* | -0.022 | 0.911 | -0.170 | 0.369 | 0.027 | 0.884 | 0.197 | 0.265 | 0.045 | 0.801 |
| **Calcium** | 0.057 | 0.757 | 0.158 | 0.414 | -0.240 | 0.201 | 0.091 | 0.621 | -0.036 | 0.840 | 0.119 | 0.502 |
| **Phosphor** | -0.270 | 0.135 | 0.004 | 0.982 | 0.025 | 0.897 | 0.115 | 0.530 | -0.137 | 0.441 | 0.179 | 0.310 |
| **APTT** | -0.101 | 0.583 | 0.073 | 0.705 | -0.259 | 0.166 | 0.017 | 0.929 | 0.163 | 0.356 | -0.387 | 0.024* |
| **D-dimer** | 0.073 | 0.692 | -0.099 | 0.610 | -0.074 | 0.697 | 0.067 | 0.715 | -0.086 | 0.630 | 0.014 | 0.938 |
| **Fibrinogen** | -0.175 | 0.338 | -0.223 | 0.245 | -0.238 | 0.205 | 0.275 | 0.128 | 0.115 | 0.517 | -0.050 | 0.780 |
| **PT** | -0.113 | 0.538 | -0.316 | 0.095 | -0.089 | 0.641 | 0.122 | 0.506 | -0.038 | 0.833 | 0.071 | 0.691 |

Spearman correlation, AML: Acute Myeloid Leukemia, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase
LDH: Lactate Dehydrogenase, APTT: Activated Partial Thromboplastin Time, PT: Prothrombin Time, *$p<0.05$

## Discussion

The present study aimed to evaluate the levels of TAS, TOS, oxidative stress and DNA damage in newly diagnosed AML patients and AML patients in remission. The results of our study demonstrated that the levels of oxidative stress and DNA damage were significantly higher in newly diagnosed and in remission AML patients compared to healthy controls. Furthermore, the DNA damage observed in newly diagnosed AML patients was found to be more pronounced than in AML patients in remission. Nevertheless, no notable discrepancy was observed between the patient and remission groups in terms of oxidative stress and antioxidant parameters.

The results of our study demonstrate a notable oxidative stress burden in newly diagnosed and remission AML patients. Our findings suggest that the reduction in antioxidant capacity may be a primary contributor to this phenomenon. Naz et al. [9] evaluated total antioxidant status (TAS) in the serum of patients with acute leukemia at the time of diagnosis, in the induction phase following remission and in healthy controls. While TAS levels were elevated at the time of diagnosis in AML, they observed that TAS levels returned to normal after the remission induction phase. In contrast to the study, our investigation revealed a notable decline in TAS levels during the remission phase. The significant reduction in total antioxidant capacity observed in patients in remission may be attributed to the decline in antioxidant enzyme levels induced by antineoplastic agents [9].

In a separate study conducted by Zhou et al. [8], oxidative stress parameters were examined in patients with newly diagnosed AML and in those who had experienced a relapse. The results indicated a significant correlation between the frequency of relapse and oxidative stress, suggesting that oxidative stress may be a prognostic factor in AML [8]. The total antioxidant levels were found to be significantly reduced in the relapse group. In particular, the levels of advanced oxidation protein products (AOPP), malondialdehyde and 8-hydroxydeoxyguanosine were significantly elevated in the recurrence group. These findings indicate that oxidative stress may serve as a promising prognostic indicator in AML, potentially influencing the progression and recurrence of the disease.

Another parameter that was subjected to analysis during our study was DNA damage or genotoxicity. The extent of DNA damage was quantified using the Comet assay. The results demonstrated that the level of DNA damage was higher in the group of patients diagnosed with AML at the time of initial presentation compared to those in remission. A paucity of data in the literature exists concerning DNA damage in AML patients. Considering the findings of our study, it can be posited that the observed abnormalities in oxidative stress and DNA damage parameters in AML patients may contribute to the disease's pathophysiology. The data presented here may be of significance in elucidating the potential association between these changes and the pathogenesis of AML.

Oxidative stress is a major contributor to various pathological conditions. Thioredoxin-1 (TXN) plays a pivotal role in the elimination of ROS, activation of tumor suppressor genes and DNA repair enzymes, which collectively contribute to maintaining cellular homeostasis. In the literature, Kamal et al. [10] conducted an evaluation of TXN gene expression in adult patients with AML and investigated its relationship with oxidative DNA damage. The researchers evaluated DNA damage by measuring serum 8-OHdG and conducting a comet assay. The expression level of TXN was found to be negatively correlated with serum 8-OHdG and tail moment in AML, but this correlation was no longer evident in relation to treatment outcome. The investigators postulated that TXN expression was inhibited in adult acute leukemia, which increases oxidative DNA damage and thus mutagenesis. The present study did not observe a greater incidence of DNA damage in the remission period when compared to the newly diagnosed AML patient group.

In conclusion, an examination and evaluation of the literature suggests that DNA damage repair mechanisms are negatively affected in AML patients consistently supporting our data. It would be prudent to consider this issue in further

studies and treatment protocols investigating the role of anti-oxidants in the treatment of acute myeloid leukemias.

In conclusion, the evidence indicates that oxidative stress and genotoxicity are increased in newly diagnosed patients and in those who have achieved remission from AML.

**Funding:** This study was supported by funding from Pamukkale University (project number: 2021HZDP008).

**Informed consent:** Written informed consent for participation and publishing of the results was obtained from all patients.

**Authors' contributions:** G.A.C., B.U.K., M.N.B., E.K.T., A.C. and M.K. designed the study. G.A.C. collected all the data. G.A.C., F.A., M.T.A., E.K.T. and A.C. carried out the data analysis and interpretation. G.A.C. and F.A. wrote and prepared the original drafts. All authors revised the drafts. All authors were involved in drafting the article or revising it critically for intellectual content, and all read and approved the final version of the manuscript.

**Conflicts of interest:** The authors declare no conflicts of interest.

## References

1. Saultz JN, Garzon R. Acute Myeloid Leukemia: A Concise Review. *J Clin Med*. 2016;5(3):33. doi:10.3390/jcm5030033
2. Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. *Haematologica*. 2012;97(12):1916-1924. doi:10.3324/haematol.2012.066100
3. Finn L, Dalovisio A, Foran J. Older Patients With Acute Myeloid Leukemia: Treatment Challenges and Future Directions. *Ochsner J*. 2017;17(4):398-404.
4. Park S, Cho BS, Kim HJ. New agents in acute myeloid leukemia (AML). *Blood Res*. 2020;55(1):14-18. doi:10.5045/br.2020.S003
5. Zhang K, Ping L, Du T, et al. A Novel Systematic Oxidative Stress Score Predicts the Prognosis of Patients with Operable Breast Cancer. *Oxid Med Cell Longev*. 2021;2021:9441896. doi:10.1155/2021/9441896
6. Chen YF, Liu H, Luo XJ, et al. The roles of reactive oxygen species (ROS) and autophagy in the survival and death of leukemia cells. *Crit Rev Oncol Hematol*. 2017;112:21-30. doi:10.1016/j.critrevonc.2017.02.004
7. Azqueta A, Ladeira C, Giovannelli L, et al. Application of the comet assay in human biomonitoring: An hCOMET perspective. *Mutat Res Rev Mutat Res*. 2020;783:108288. doi:10.1016/j.mrrev.2019.108288
8. Zhou FL, Zhang WG, Wei YC, et al. Involvement of oxidative stress in the relapse of acute myeloid leukemia. *J Biol Chem*. 2010;285(20):15010-15015. doi:10.1074/jbc.M110.103713
9. Naz A, Shamsi TS, Sattar A, Mahboob T. Oxidative stress and total antioxidant status in acute leukemia at diagnosis and post remission induction phase. *Pak J Pharm Sci*. 2013;26(6):1123-1130.
10. Kamal AM, El Hefny NH, Hegab HM, El Mesallamy HO. Expression of thioredoxin-1 (TXN) and its relation with oxidative DNA damage and treatment outcome in adult AML and ALL: A comparative study. *Hematology*. 2016;21(10):567-575. doi:10.1080/10245332.2016.1173341