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**RESEARCH**

# Assessment of genotoxic and cytotoxic effects in COVID-19 patients

## COVID-19 hastalarında genotoksik ve sitotoksik etkilerin değerlendirilmesi

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**Abstract**

**Purpose:** The objective of our study was to ascertain whether the SARS-CoV-2 virus exerts direct cytotoxic and genotoxic effects on human blood defence cells.

**Materials and Methods:** An *in vitro* analysis was conducted to assess the cytotoxic and genotoxic effects of the virus using three established tests: the mitotic index (MI), micronucleus (MN), and comet assay (CA). These tests were applied to blood samples from 101 patients. The blood samples were simultaneously analyzed using the polymerase chain reaction (PCR) test. The study population included patients of all ages and genders who presented to the outpatient clinic with symptoms suggestive of a respiratory tract infection and fever.

**Results:** The frequency of MN in the human lymphocytes of COVID-19-infected patients (1.06) was higher compared to COVID-19-negative patients (0.68). Similarly, in COVID-19-positive individuals, parameters such as tail length (3.67), tail moment (1.786), and tail intensity in the comet assay showed a significant increase compared to the negative control, indicating DNA damage. In the cytotoxicity assessment, the MI frequency of COVID-19-positive individuals (0.041) was significantly lower than that of negative controls (0.051). Gender did not influence the cyto/genotoxicity (except for tail length) in SARS-CoV-2-infected patients. Among age groups, the SARS-CoV-2 virus increased MI frequency and tail intensity only in middle-aged individuals (26–36 years).

**Conclusion:** The SARS-CoV-2 virus has the potential to induce cytotoxic and genotoxic effects in the human lymphocytes of infected individuals.

**Keywords:**. Comet assay, COVID-19, genotoxicity, micronucleus, mitotic index

**Öz**

**Amaç:** Çalışmamızda COVID-19 virüsünün insan lenfositleri üzerindeki doğrudan sitotoksik ve genotoksik etkisinin olup olmadığının saptanması amaçlanmıştır.

**Gereç ve Yöntem:** Virüsün sitotoksik/genotoksik etkileri 101 hastadan alınan kan örnekleri ile *in vitro* mitotik indeks (MI), Mikronükleus (MN) ve Comet Assay (CA) testleri kullanılarak değerlendirilmiştir. Hastalardan alınan kan örnekleri PCR testi ile eş zamanlı olarak analiz edilmiştir. Çalışmaya COVID-19 polikliniğine üst solunum yolu ve ateş şikayetleriyle başvuran her yaş grubundan ve cinsiyetten hastalar dahil edilmiştir.

**Bulgular:** COVID-19 ile enfekte olmuş hastaların insan lenfositlerindeki MN sıklığı (1.06) COVID-19 negatif hastalara (0.68) kıyasla artmıştır. Benzer şekilde COVID-19 pozitif bireylerde komet testindeki kuyruk uzunluğu (3.67), kuyruk momenti (1.786) ve kuyruk yoğunluğu parametreleri negative kontrole kıyasla önemli ölçüde artış göstererek DNA hasarına neden olmuşlardır. Sitotoksisite değerlendirmesinde COVID-19 pozitif bireylerin MI frekansı (0.041) negatiflere (0.051) göre anlamlı derecede düşmüştür. Cinsiyet SARS-CoV-2 enfekte hastalarda sito/genotoksisiteyi (kuyruk uzunluğu hariç) etkilememiştir. Yaş gruplarında, SARS-CoV-2 virüsü MI sıklığını ve kuyruk yoğunluğunu yalnızca orta yaşta (26-36) artırmıştır.

**Sonuç:** SARS-CoV-2 virüsü, COVID-19 ile enfekte hastalarda insan lenfositleri üzerinde sitotoksik ve genotoksik etkilere neden olabilir.

**Anahtar kelimeler**: Comet assay, COVID-19, genotoksisite, mikronükleus, mitotik indeks.

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## INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the disease known as coronavirus disease 2019 (COVID-19). This disease was first identified in Wuhan, China, in December 2019 and subsequently spread rapidly from person to person, resulting in a global pandemic[1]. Since the first cases were reported in China, the virus has mutated rapidly into multiple variants, gaining increased transmissibility and the ability to evade the immune response. While the immediate concern with COVID-19 often centers on its respiratory symptoms, its toxicological effects on human health extend far beyond the respiratory system[2,4].

The existing literature on human genetic factors associated with the incidence, morbidity, and mortality of the disease caused by the SARS-CoV-2 coronavirus indicates notable differences related to gender, ABO blood groups, human leukocyte antigen (HLA) genotypes, population groups, ethnicities, and geographic backgrounds with regard to these factors[5-8].

Viruses possess a remarkable ability to infiltrate host cells and manipulate their biological processes. Beyond the immediate symptoms and pathogenesis of viral infections, lesser-known but equally important aspects of cytotoxicity and genotoxicity come into play. The damage induced by viral infections can involve both direct cell death and genetic alterations. Notable examples include Human Papillomavirus (HPV), Hepatitis B/C, and Epstein-Barr Virus, which are associated with carcinoma due to the genotoxic effects of viral replication[9].

The assessment of genotoxic and cytotoxic effects on human peripheral blood lymphocytes is a standardized methodology employed in toxicological and environmental health investigations. Genotoxicity and cytotoxicity assessments are crucial for understanding potential damage to the genetic material of peripheral blood lymphocytes, which are key players in the immune system.

Such studies in the field of toxicology and environmental health measure the impact of various agents, including radiation, chemicals, and environmental stressors, on human health[10-18]. Genotoxicity refers to the ability of a substance to cause damage to an organism's genetic material, particularly DNA. Basic assays, such as the comet assay (CA) and the micronucleus (MN) assay, are commonly used to evaluate genotoxic effects on human peripheral blood lymphocytes[19]. The MN method indicates genotoxicity by detecting the presence of small additional nuclei, called micronuclei, which are formed during cell division when chromosomes or chromosomal fragments fail to be incorporated into the main nucleus[20].

The comet assay (CA) is a simple, rapid, and widely used technique for assessing DNA breakage at the single-cell level by embedding lymphocytes in an agarose gel. Damaged DNA migrates further, creating a "comet tail." Measuring the length and intensity of the tail reveals the extent of DNA damage[21].

The mitotic index (MI) is a metric that provides valuable information about the growth and proliferation dynamics of cells by quantifying the rate of cell division. It is commonly used in histopathology, cell biology, and cancer research[22].

Understanding the cytotoxic and genotoxic effects of viral diseases is crucial for clinical management and scientific research. It underscores the urgent need for the development of antiviral therapies, vaccines, and strategies to mitigate the detrimental consequences of viral infections.

Many studies in the literature have focused on the SARS-CoV-2 virus and the disease it causes, which has persisted as a pandemic for over two years and continues to cause occasional outbreaks. However, when examining the literature from this perspective, no studies were found addressing the genotoxic or cytotoxic effects of the SARS-CoV-2 virus on human lymphocytes. In this article, we delve into the toxicological impact of the virus on the human body and explore the implications for patient care and ongoing research. The aim of this study is to evaluate the cytotoxicity and genotoxicity of the SARS-CoV-2 virus in human lymphocytes using CA, MN, and MI tests.

## MATERIALS AND METHODS

### Study design

The study was conducted at an outpatient clinic dedicated to the treatment of patients infected with the SARS-CoV-2 virus. It adhered to the guidelines established by the Clinical Research Ethics Committee of Çanakkale 18 Mart University (decision number: 06-14, decision date: 04/06/2022).

Blood samples were collected at the Infectious Diseases Unit of ÇOMÜ Research Hospital, PCR results were evaluated, and genotoxic/cytotoxic analyses of the samples were performed at the Genetics Laboratory of the Vocational School of Health Services. The study population consisted of patients of all ages and genders who presented with upper respiratory tract symptoms and fever. Before participation, all subjects provided informed consent. A total of 101 blood samples were collected, of which 32 (31.7%) tested negative for SARS-CoV-2 by PCR analysis. The remaining 69 participants (68.3%) tested positive for the virus. Among the SARS-CoV-2-positive participants, 67 (66.3%) were female, and 34 (33.7%) were male.

## Sample

Power analysis was conducted using the G*Power program. It was determined that a study should include a minimum of 100 participants divided into two groups, with a confidence level of 95% (1-α), a test power of 80% (1-β), and an effect size of d=0.51 (large effect size).

Eligibility criteria for the study required participants to be 18 years of age or older and to have undergone a PCR test. Exclusion criteria included age incompatibility, exposure to radiation within the last six months, receiving immunosuppressive treatment, chemotherapy, or radiotherapy, and being hospitalized for treatment. Participants who did not provide consent were also excluded. Participants were divided into three age groups: 18–25, 26–36, and 37+. Of the participants, 55 (54.5%) were non-smokers, while 46 (45.5%) were smokers. Regarding alcohol consumption, 73 participants (72.3%) did not consume alcohol, while 28 participants (27.7%) did.

Among all participants, 19 (18.8%) had chronic diseases, while 82 (81.2%) did not. Similarly, 82 participants (81.2%) were not on medication, whereas 19 (18.8%) were taking medication. Of the participants, 37 (36.6%) had a history of Covid-19, while 64 (63.4%) had never contracted the virus. In terms of vaccination, 94 participants (93.1%) were vaccinated against Covid-19, while 7 (6.9%) were not. Among the vaccinated participants, 28 (27.7%) received the Sinovac vaccine, and 66 (65.3%) received the BioNTech vaccine.

## Procedure

In the study, evaluations were completed by performing MI, MN, and comet analyses for a total of 101 individuals. The results of each test were compared based on the participants' COVID-19 status, gender, age, smoking habits, and previous COVID-19 experience. These comparisons were used to evaluate the genotoxic and cytotoxic effects.

### Mitotic index test

Chromosome Medium B was distributed into sterile tubes in 2.5 mL volumes. Blood samples from 101 individuals in the study group were added to the medium (Chromosome Medium B) in 0.2 mL amounts, with heparin added at a 1:10 ratio. COVID-positive and -negative patients were studied separately. The cells were cultured at 37°C for 72 hours.

At the 70th hour of the study, a dose of 0.06 μg/mL colchicine was added to the culture. Upon completion of the culture period, the tubes were centrifuged at 1200 rpm for ten minutes. The cultured cells were treated with a hypotonic solution of 0.075 M KCl for 30 minutes at a temperature of 37°C, followed by fixation with cold methanol and acetic acid in a ratio of 3:1. The cells were subjected to three rounds of fixation.

The slides were stained with 5% Giemsa (pH 6.8) prepared in Sorensen buffer for 20–25 minutes. They were then washed in distilled water, dried at room temperature, and mounted with Depex. To ascertain the MI, a total of 6,000 cells were examined for each dose, with 1,500 cells examined for each individual across all applications. To calculate the MI, it is first necessary to divide the number of cells that have undergone cell division by the total number of cells and then express the result as a percentage.

### Micronucleus test method

As in the MI test method, 0.2 mL blood samples taken from a total of 101 COVID-positive and COVID-negative individuals were transferred to culture tubes containing 2.5 mL of medium. Lymphocytes were incubated at 37°C for 72 hours. Cytochalasin B (5.2 mg/mL) was added to block cytokinesis 28 hours before the end of the culture. Cells were centrifuged (1000 rpm for 10 minutes), and pellets were treated with a hypotonic solution (0.075M KCl) for 5 minutes at 4°C. Cells were re-centrifuged and fixed three times in a 3:1 cold fixative. In the last fixation, 5 mL of fixative, obtained by adding 1% formaldehyde to the 3:1 fixative, was added to each tube. To preserve the cytoplasm, 1%

formaldehyde was added to the final fixative. Slides were prepared by dehydration and air drying. They were stained with 5% Giemsa (pH 6.8) in Sorensen's buffer for 13 to 15 minutes, rinsed in distilled water, dried at room temperature, and mounted with Depex. In permanent preparations, MN frequencies were determined in 1,000 binucleate cells for each individual for each dose (4,000 binucleate cells in total for each dose).

### Comet assay

The CA was conducted under alkaline conditions. Peripheral blood taken from the patients was mixed with PBS in the tube (900 µL PBS + 100 µL blood). Blood and PBS were suspended and kept on ice for 10 minutes. Then, 100 µL of LymphoPrep was added to each tube and centrifuged at 1060 rpm at +4°C for 3 minutes. Lymphocytes were then obtained. Meanwhile, trypan blue was used to detect the viability of the cells. The lymphocytes obtained were distributed into Eppendorf tubes as 100 µL each and cultured at 37°C for 1 hour. After culture, the Eppendorfs were centrifuged at 3000 rpm for 5 minutes. After centrifugation, the supernatant was discarded and resuspended by adding 100 µL PBS. Then, 100 µL of lymphocytes was mixed with Low Melting Agar (100 µL) and spread on slides previously covered with agar. The slides were covered with coverslips and kept at +5°C for 20-25 minutes. At the end of the period, the coverslips on the slides were removed and kept at +5°C for 1 hour during the lysing process. Then, the slides were placed in the electrophoresis tank and kept in electrophoresis buffer for 20 minutes. At the end of the period, the preparations were run. After electrophoresis, the slides were kept in neutralization buffer and kept at +4°C for 10 minutes. At the end of the procedures, staining was done with 50 µL EtBr and examined under a microscope with a fluorescent attachment at 40X magnification. 100 cells from each donor were examined, and the results were evaluated in terms of % tail density, tail length, and tail moment.

### Statistical analysis

In the analysis of the data obtained in the study, SPSS version 18 program [Statistical Packages for the Social Sciences (SPSS) version 18 commercial software (IBM Corp.; Armonk, NY, USA)] was used to perform normality of numerical variables, Kolmogorov Smirnov test, Histogram graph, skewness and kurtosis tests. Since the normality condition was met, independent samples t test and one-way Anova test were applied, and 0.05 was taken as the significance level.

## RESULTS

In the study, 32 of the 101 people whose blood was taken were Covid positive (68.3%), while 69 were Covid negative (31.7%). Participants in the study, 67 were women (66.3%) and 34 were men (33.7%), and they were divided into three age groups: 18-25, 26-36 and 37+. The number of individuals in these groups is 28, 45 and 28, respectively. 55 (54.5%) of the participants included in the study were non-smokers, and 46 (45.5%) were active smokers. The number of participants who did not consume alcohol was 73 (72.3%), and the number of participants who did use alcohol was 28 (27.7%). While 19 (18.8%) of the participants had a chronic disease (hypertension, diabetes, celiac disease, thyroid disorders, thalassemia), 82 (81.2%) did not. Similarly, 82 (81.2%) people were not using any medication, while 19 (18.8%) were receiving medication (antipsychotic medication, thyroid hormone medication, blood pressure medication, insulin). While the number of patients previously infected with COVID-19 was 37 (36.6%), the number of patients who had never been infected was 64 (63.4%). While the number of people vaccinated against COVID-19 was 94 (93.1%), the number of people who were not vaccinated at all was 7 (6.9%). Among those vaccinated, the rate of those vaccinated with Sinovac was 28 (27.7%) and the number of those vaccinated with BioNTech was 66 (65.3%) (Table 1).

In the MN and MI study, the number of COVID-19 negative female individuals was 43 (62.3% of negative individuals), while the number of Covid-19 negative male individuals was 26 (26.7% of negative individuals). The number of Covid-19 positive male individuals was 8 (25% of positive individuals), and the number of Covid-19 positive female individuals was 24 (75% of positive individuals).

In this study, MI, MN, and CA (tail length, tail density, and tail moment) results of covid positive and covid negative patients were compared. These comparisons are statistical comparisons of situations such as gender, age, smoking or not.

The MI frequencies were 4.1% in Covid-positive patients, while they were 5.1% in negative patients. This difference is statistically significant. It was determined that the MN frequency was 1.059% in Covid-positive patients and 0.678 in Covid-negative

patients. This increase in Covid-positive individuals is statistically significant compared to Covid-negative individuals (p = 0.002). It has been determined that there are increases in the frequency of Comet parameters in Covid-positive patients. Statistically significant differences were found in all three parameters in the evaluation of Comet tail length, tail density and tail moment in Covid-positive patients compared to Covid-negative patients (Table 2).

**Table 1. Socio-demographic and introductory characteristics of participants (n=101)**

| **Variables** | **Groups** | | |
|---|---|---|---|
| **Age** | **X̄±sd** | **min** | **max** |
| | 32.82±12.22 | 18 | 81 |
| | | n | % |
| Covid Situation | Negative | 69 | 68.3 |
| | Positive | 32 | 31.7 |
| Gender | Female | 67 | 66.3 |
| | Male | 34 | 33.7 |
| Age Groups | 18-25 | 28 | 27.7 |
| | 26-36 | 45 | 44.6 |
| | 37+ | 28 | 27.7 |
| Smoking Status | No | 55 | 54.5 |
| | Yes | 46 | 45.5 |
| Alcohol Consumption Status | No | 73 | 72.3 |
| | Yes | 28 | 27.7 |
| Chronic Disorder Status | No | 82 | 81.2 |
| | Yes | 19 | 18.8 |
| Chronic Drug Use Status | No | 82 | 81.2 |
| | Yes | 19 | 18.8 |
| Previous COVID Experience | No | 64 | 63.4 |
| | Yes | 37 | 36.6 |
| Vaccination Status | No | 7 | 6.9 |
| | Sinovac | 28 | 27.7 |
| | Biontech | 66 | 65.3 |

**X̄**: Average, sd: Standard deviation

The evaluation of whether the gender of Covid-positive patients would differ in terms of cytotoxicity was made with the MI test. According to the results obtained, it was determined that gender did not have any effect on the change in MI frequency in Covid-positive patients. The evaluation made in terms of MN frequency determined that there were significant increases in Covid-positive patients compared to Covid-negatives in both genders. When comparing the genders with each other, there is no statistically significant difference in the increase in MN frequency in male and female individuals. Similarly, it was determined that different gender characteristics did not change the DNA damage frequency in Covid-positive patients (except tail length) (Table 3).

MI frequencies were evaluated between age groups and no statistical difference was found between these groups. In the MN test, micronucleus frequency showed a significant difference only in the middle age group (26-36) compared to other groups. There was no difference between the groups in the CA test and this increase was statistically significant (Table 4).

Another parameter used in the study is smoking. Covid-positive patients were grouped as smokers and non-smokers. It has been determined that the MN frequency decreases in individuals who smoke, but this decrease is not statistically significant. In the MI test, the frequencies of smokers and non-smokers were equal. On the other hand, in the CA test, the tail length is higher in non-smoking Covid-positive patients than in smokers, and this increase is statistically significant (Table 5).

Currently, there are 40 people (57.97%) in the Covid Negative group who have previously had COVID. The number of people who have never been Covid positive is 29 (42.03%). In the study, among Covid-negative individuals, individuals who were previously diagnosed as Covid positive (X̄: 0.75) had a higher MN frequency than those who had never been Covid

positive (0.63). But this is not statistically significant. In the MI test, this situation was equal to each other (X̄: 0.05). In the CA test, the DNA damage frequencies of those who had previously had Covid were significantly lower in tail moment and tail length parameters than those who had never been Covid positive.

**Table 2. The status of genotoxic and cytotoxic effects in covid positive and negative individuals**

| Tests | COVID Status | X̄ ± sd | t | P |
|---|---|---|---|---|
| MN Frequency % | Negative | 0.678±0.502 | 3.232 | 0.002 |
| | Positive | 1.059±0.647 | | |
| MI | Negative | 0.051±0.01 | 5.057 | >.001 |
| | Positive | 0.041±0.007 | | |
| Tail length | Negative | 3.673±1.371 | 3.532 | >.001 |
| | Positive | 4.631±1.005 | | |
| Tail Intensity | Negative | 22836.862±5412 | 3.689 | >.001 |
| | Positive | 28178.282±7313 | | |
| Tail Moment | Negative | 1.786±0.806 | 3.431 | >.001 |
| | Positive | 2.656±1.325 | | |

X̄: Mean, sd : Standard deviation

**Table 3. Assessment of COVID status by gender**

| Tests | COVID | Gender | X̄ ±sd | t | p |
|---|---|---|---|---|---|
| MN Frequency % | Negative | Female | 0.67±0.52 | 0.081 | 0.936 |
| | | Male | 0.68±0.49 | | |
| | Positive | Female | 1.05±0.71 | 0.202 | 0.841 |
| | | Male | 1.10±0.42 | | |
| MI | Negative | Female | 0.05±0.01 | 1.876 | 0.065 |
| | | Male | 0.05±0.01 | | |
| | Positive | Female | 0.04±0.01 | 0.057 | 0.955 |
| | | Male | 0.04±0.01 | | |
| Tail length | Negative | Female | 3.84±1.35 | 0.844 | 1,311 |
| | | Male | 3.40±1.38 | | |
| | Positive | Female | 4.35±0.93 | 3.078 | 0.004 |
| | | Male | 5.47±0.75 | | |
| Tail Intensity | Negative | Female | 23598.45±6363.75 | 1.781 | 0.080 |
| | | Male | 21577.32±2997.71 | | |
| | Positive | Female | 27902.75±8248.67 | 0.527 | 0.602 |
| | | Male | 29004.89±3505.69 | | |
| Tail Moment | Negative | Female | 1.78±0.72 | 0.028 | 0.977 |
| | | Male | 1.79±0.95 | | |
| | Positive | Female | 2.45±1.28 | 1.575 | 0.126 |
| | | Male | 3.28±1.33 | | |

X̄: Mean, sd: Standard deviation

**Table 4. The relationship between genotoxic and cytotoxic effects between age groups and covid status**

| Tests | COVID | Age Groups | X̄ ± sd | F | p |
|---|---|---|---|---|---|
| MN Frequency % | Negative | 18-25 | 0.62±0.59 | 1.453 | 0.241 |
| | | 26-36 | 0.6±0.48 | | |
| | | 37+ | 0.83±0.42 | | |
| | Positive | 18-25 | 1.36±0.63 | 1.273 | 0.295 |
| | | 26-36 | 0.93±0.55 | | |
| | | 37+ | 1.05±0.89 | | |
| MI | Negative | 18-25 | 0.0505±0.0120 | 0.818 | 0.446 |
| | | 26-36 | 0.0531±0.0092 | | |
| | | 37+ | 0.0496±0.0085 | | |
| | Positive | 18-25 | 0.0365± 0.0013[a] | 4.413 | 0.021 |
| | | 26-36 | 0.0442± 0.0167[b] | | |
| | | 37+ | 0.0393±0.0278 [ab] | | |
| Tail length | Negative | 18-25 | 3.88±1.33 | 0.385 | 0.682 |
| | | 26-36 | 3.52±1.31 | | |
| | | 37+ | 3.68±1.52 | | |
| | Positive | 18-25 | 5.15±0.43 | 1.490 | 0.242 |
| | | 26-36 | 4.48±1.13 | | |
| | | 37+ | 4.39±1.03 | | |
| Tail intensity | Negative | 18-25 | 25357.33± 8316.71[a] | 3.307 | 0.043 |
| | | 26-36 | 21603.88±3114.7 [b] | | |
| | | 37+ | 22058.73±3407.88 [eu] | | |
| | Positive | 18-25 | 28006.92±6435.49 | 2.184 | 0.131 |
| | | 26-36 | 26500.37±6696.37 | | |
| | | 37+ | 33440.51±8820.2 | | |
| Tail Moment | Negative | 18-25 | 2.12±0.99 | 2.532 | 0.087 |
| | | 26-36 | 1.64±0.88 | | |
| | | 37+ | 1.66±0.32 | | |
| | Positive | 18-25 | 1.94±0.94 | 1.754 | 0.191 |
| | | 26-36 | 2.97±1.51 | | |
| | | 37+ | 2.66±0.84 | | |

X̄: Mean, sd: Standard deviation. There is no difference between interactions with the same letter for each parameter.

**Table 5. The relationship between smoking and genotoxic and cytotoxic effects in Covid-positive and negative individuals**

| Tests | COVID | Smoking | X̄ ±sd | t | p |
|---|---|---|---|---|---|
| MN Frequency % | Negative | No | 0.66±0.53 | 0.478 | 0.696 |
| | | Yes | 0.71±0.47 | | |
| | Positive | No | 1.11±0.71 | 0.418 | 0.679 |
| | | Yes | 1.02±0.61 | | |
| MI | Negative | No | 0.05±0.01 | 1.314 | 0.193 |
| | | Yes | 0.05±0.01 | | |
| | Positive | No | 0.04±0.01 | 1.074 | 0.291 |
| | | Yes | 0.04±0.01 | | |
| Tail length | Negative | No | 3.79±1.52 | 0.878 | 0.383 |
| | | Yes | 3.5±1.13 | | |
| | Positive | No | 5.07±0.83 | 2.365 | 0.025 |
| | | Yes | 4.29±1.02 | | |
| Tail intensity | Negative | No | 24191.72±6025.7 | 2.623 | 0.011 |
| | | Yes | 20852.96±3620.43 | | |
| | Positive | No | 28615.93±7075.01 | 0.294 | 0.771 |
| | | Yes | 27837.89±7680.23 | | |
| Tail Moment | Negative | No | 1.81±0.82 | 0.253 | 0.801 |
| | | Yes | 1.76±0.81 | | |
| | Positive | No | 2.43±0.95 | 0.997 | 0.377 |
| | | Yes | 2.83±1.56 | | |

X̄: Mean, sd: Standard deviation

**Table 6. Genotoxic/cytotoxic evaluation of individuals based on their COVID-19 history**

| Tests | Current COVID status | Previously COVID situation | X̄±sd | t | p |
|---|---|---|---|---|---|
| MN Frequency % | Negative | No | 0.63±0.46 | 1.036 | 0.304 |
| | | Yes | 0.75±0.55 | | |
| | Positive | No | 1.08±0.66 | 0.233 | 0.817 |
| | | Yes | 1.01±0.66 | | |
| MI | Negative | No | 0.05±0.01 | 0.887 | 0.378 |
| | | Yes | 0.05±0.01 | | |
| | Positive | No | 0.04±0.01 | 1.223 | 0.231 |
| | | Yes | 0.04±0.01 | | |
| Tail length | Negative | No | 4.05±1.29 | 2.770 | 0.007 |
| | | Yes | 3.16±1.34 | | |
| | Positive | No | 4.41±1.07 | 3.531 | 0.001 |
| | | Yes | 5.28±0.32 | | |
| Tail intensity | Negative | No | 22025.3±4827.34 | 1.476 | 0.145 |
| | | Yes | 23956.26±6036.27 | | |
| | Positive | No | 27134.22±7002.86 | 0.833 | 0.165 |
| | | Yes | 31310.46±7798.21 | | |
| Tail Moment | Negative | No | 1.96±0.85 | 2.120 | 0.038 |
| | | Yes | 1.55±0.69 | | |
| | Positive | No | 2.48±1.32 | 1.347 | 0.188 |
| | | Yes | 0.63±0.46 | | |

X̄: Mean, sd: Standard deviation

## DISCUSSON

Recent advances in molecular cancer genetics have shown that most carcinogens are genotoxic, and carcinogenesis is associated with mutations in oncogenes and antioncogenes. Genotoxicity tests are mainly used in cancer prevention, investigating the effects of environmental factors and industrial chemicals, and investigating the toxic effects and safety of drugs before they are put on the market. From the 1970s to the present, many *in vivo* and *an in vitro* genotoxicity tests have been developed[23]. Although the studies about the cytotoxicity and genotoxicity effects of radiation, chemicals, environmental stressors, and various factors have been conducted in the field of toxicology environmental health, it has come to our attention that virus studies have not been enough carried out. Since currently the CA has been extensively used in toxicological genetics studies, also it has been used in virology studies to help understand the mechanisms behind viral oncogenesis[24-28]. The assay has also revealed the genotoxic effects of viruses such as measles virus and bovine leukaemia virus[29,30]. Therefore, the CA can be considered a reliable and accurate method to investigate the oncogenic process associated with viral infections. In this study, in addition to the CA test, MI and MN tests were applied to 101 individuals.

The increase in the frequency of some types of cancer is caused by infectious diseases[31,32] The International Agency for Research on Cancer (IARC) has reported 10 pathogens, including viral infections, as group 1 carcinogens[33]. It is known that approximately 12% of human cancer types develop because of viral infections. These cancers, which are caused by viruses, occur mainly in people whose immune systems are weakened or compromised[34]. Interestingly, immune dysfunction is often associated with the risk factors associated with severe COVID-19 patients, such as obesity and T2D.

Viral infections can cause DNA damage and genotoxic effects by encoding oncogenic viral proteins, causing chronic inflammation, and causing genotoxic damage[35]. The results in MN, MI and CA are parallel to those mentioned above. The results obtained show that the cytotoxic and genotoxic effect increases in COVID-19 positive individuals[36].

The biology of SARS-CoV-2 is not fully understood, but it is thought to behave similarly to coronaviruses such as the infectious bronchitis virus (IBV), triggering molecular mechanisms akin to those of IBV. Non-structural proteins like nsp13 from SARS-

CoV and IBV have been reported to interact with DNA polymerase δ, leading to DNA replication fork stress, DNA damage, H2AX histone phosphorylation, and cell cycle arrest[37]. The nsp13 proteins of SARS-CoV and SARS-CoV-2 share 99.8% similarity, indicating that genotoxic and cytotoxic mechanisms induced by SARS-CoV-2 coronavirus may similarly occur or be induced[38].

Replication fork defects induced by nsp13 increase genetic instability, promoting tumorigenesis[39]. DNA polymerase δ is involved in several DNA repair mechanisms and is one of the most important enzymes for genomic stability[40-41 ,44,45]. Thus, in addition to directly contributing to DNA damage caused by replication fork stress, the effects of NSP-13 on DNA polymeraseδ may also contribute to genome instability in the presence of xenobiotics and various environmental factors. In our study, the increase in Comet tail intensity (28178), tail length (4.631), and tail moment (2.656) observed in the comparison of DNA damage frequency between Covid-positive individuals and Covid-negative individuals could be attributed to the nsp13 protein.

The genotoxic and cytotoxic effects of the drugs used against SARS-CoV 2 virus have been studied. Examples of these are drugs such as molnupiravir, favipiravir and resveratrol. Favipiravir and resveratrol exhibited a strong cytotoxic and genotoxicity activity in cells[42-45]. In Favipiravir related genotoxicity study has showed DNA damage in cardiomyoblast cells and fibroblastic skin cells by using the CA and detecting the increase in DNA tail[46]. According to these studies, whether patients use medication is important in determining the virus-induced cytotoxic and/or genotoxic effect in Covid-positive patients. In patients taking medication, the effects of the medication and the virus may cause a cumulative effect. In this study, MN frequency of individuals who had a negative COVID test and did not use medication was determined to be 0.51%. On the other hand, the MN frequency of individuals who did not use medication but had a positive COVID test was found to be twice as high as the other and was determined as 1.26%. These results indicate that the SARS-CoV 2 virus may trigger clastogenic and/or anogenic effects.

Scientifically, studies on the genotoxic-cytotoxic effect of the SARS-CoV 2 virus are limited in number. Gonçalves et al. aimed to evaluate the possible induction of mutagenic (via MN test) and genotoxic (CA) effects in Poecilia reticulata adults exposed to fragments of the Spike protein of SARS-CoV-2, denominated PSPD-2002. According to this study PSPD-2002 peptides were able to cause genomic instability and erythrocyte DNA damage[47]. Our results also parallel to this study.

The study limitations include the fact that there were 37 Covid-19 positive individuals in the study and that COVID-19 positive/COVID-19 negative individuals with respiratory complaints could not be compared with healthy individuals with no complaints (control group) separately. Blood samples were taken from 101 individuals who had a PCR test for the study and met the study criteria at different times and Comet, MN and MI tests were performed on the day of blood collection. 37 of these individuals were found to be COVID-19 positive in the PCR test. Due to the fading of the pandemic, the number of cases remained limited to 37 due to the rarity of COVID-19 positive cases.

In conclusion, genotoxic/cytotoxic frequency was evaluated in COVID-19 infected patients with three different test methods such as MN, MI, and single cell gel electrophoresis, which has been approved by international organizations and has validity still ongoing in this study. In this context, it was determined that the frequency of MN, MI and tail moment, tail intensity and tail length in human lymphocytes of patients infected with COVID-19 was increased compared to COVID-19 negative patients. The gender, age, smoking generally did not affect genotoxicity and cytotoxicity in COVID- 19 infected patients. Within the concentration range used in this study cytotoxic and genotoxic effects detected in human lymphocytes in COVID-19 infected patients. It is important to increase *in vivo/in vitro* studies to fully reveal the genotoxic and cytotoxic profile of SARS-CoV-2.

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